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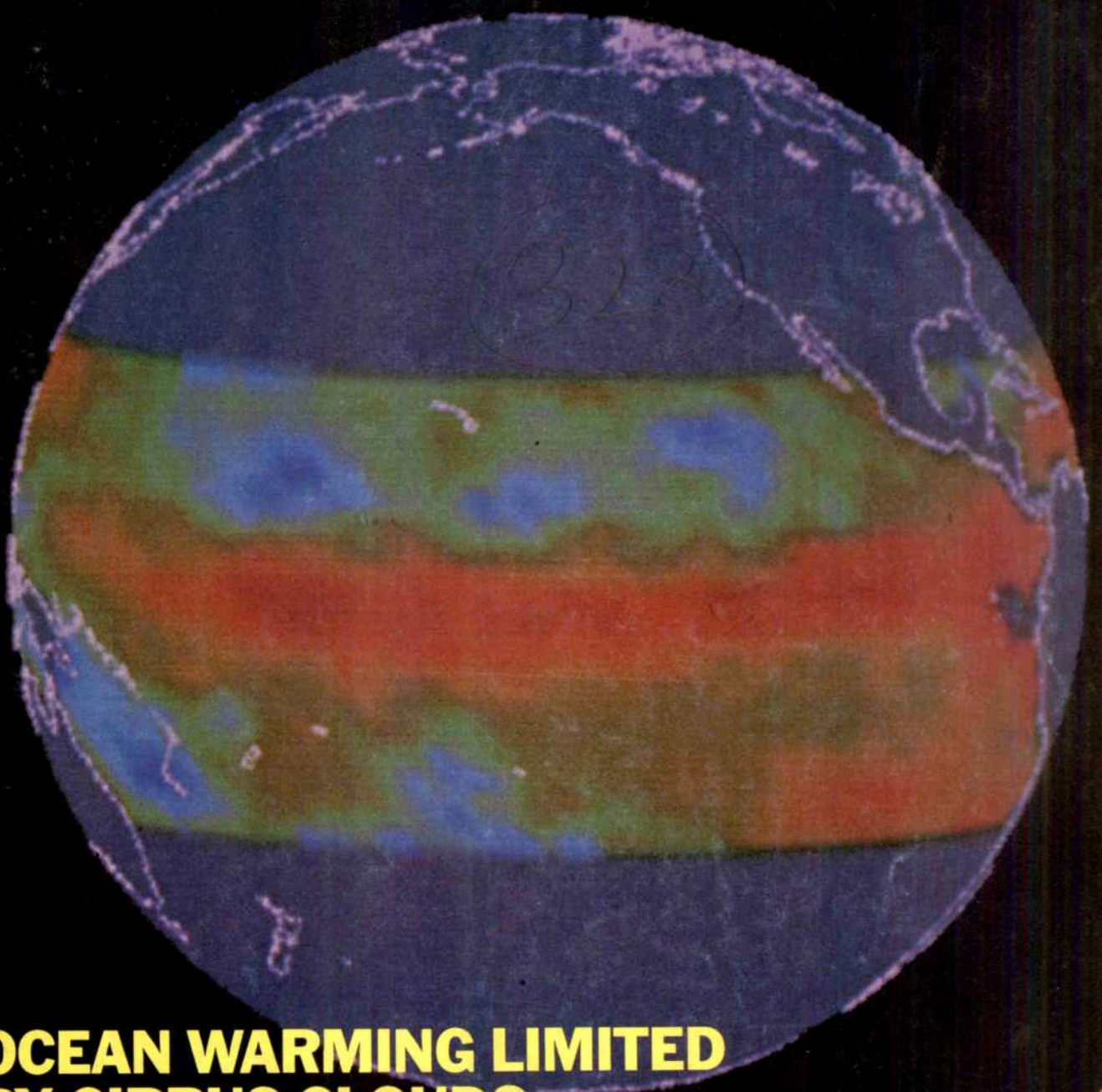

OCEAN WARMING LIMITED BY CIRRUS CLOUDS

Mating competition and sexual selection

Mechanism of deep earthquakes

MICROBIOLOGY

Southerns/Northerns: Electrophoresis, Blotting, and Crosslinking in 2.5 Hours Instead of 30.

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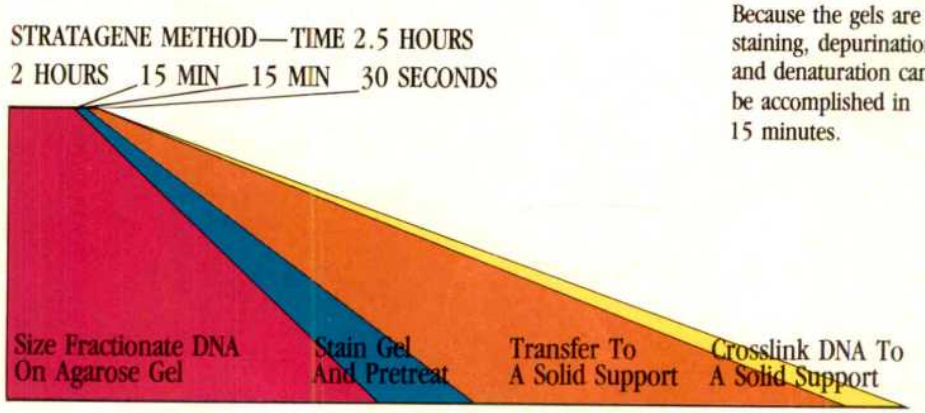


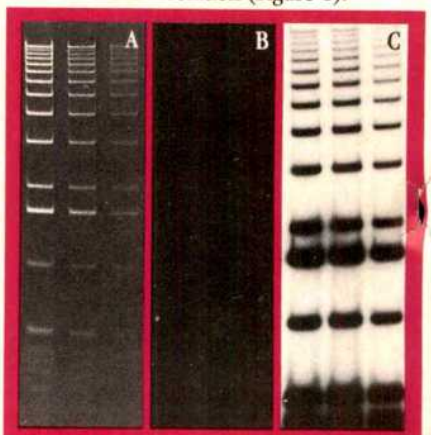
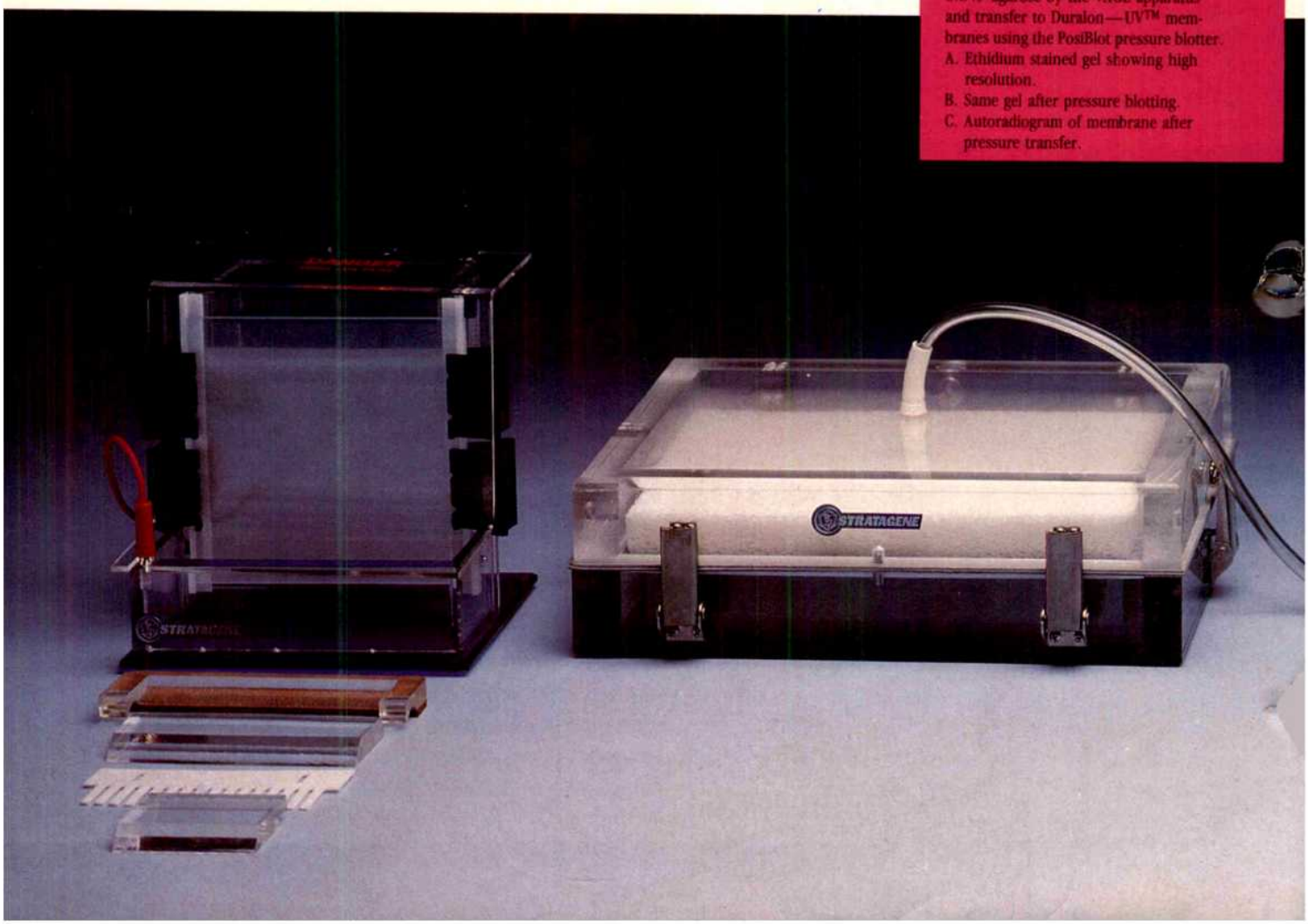


FIGURE 1:
Figure Legend: Fractionation of end labeled DNA markers on 3mm thick 0.8% agarose by the VAGE apparatus and transfer to Duralon—UV™ membranes using the PosiBlot pressure blotter.
A. Ethidium stained gel showing high resolution.
B. Same gel after pressure blotting.
C. Autoradiogram of membrane after pressure transfer.



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PosiBlot™* Pressure Blotter

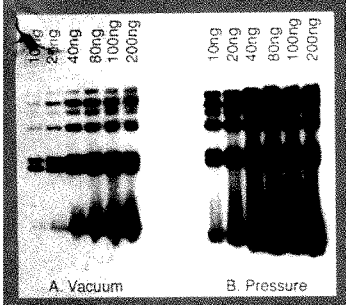

FIGURE 2:
Figure Legend: ^{32}P end-labeled lambda Hind III markers were electrophoresed in 0.8% agarose. The DNA was then transferred to a nylon membrane with a vacuum blotter at 30mm Hg below atmospheric or with the PosiBlot pressure blotter at 100mm Hg above atmospheric. Both transfers were carried out for 15 minutes. As can be seen, pressure blotting transferred significantly more DNA in the same period of time, especially in the higher molecular weight range (largest band is 23 kilobases).

The PosiBlot™ positive pressure blotter permits the transfer of nucleic acids in 1/3 the time of vacuum blotters and 1/50 the time of capillary blotting (Figure 2). Pressure blotting does not dehydrate gels as do other methods. This allows the use of substantially higher pressure differentials, compared with vacuum blotting, without gel collapse. The PosiBlot apparatus reduces blotting time to 15 minutes.

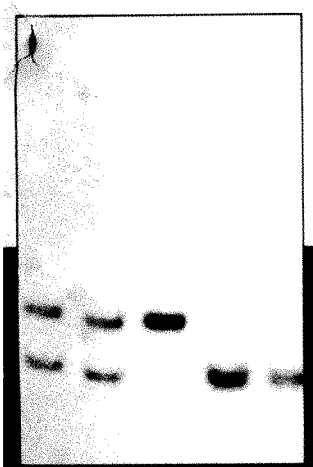

FIGURE 3:
Figure Legend: Autoradiogram showing the resolution of 2.8 and 1.3 Kb Msp I RFLP alleles revealed by a cystic fibrosis human DNA probe using the VAGE, PosiBlot and Stratalinker all in 2.5 hours.

Stratalinker™* UV Crosslinker

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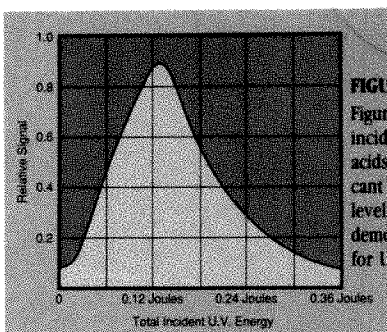

FIGURE 4:
Figure Legend: The effects of altering the incident energy for crosslinking nucleic acids to nylon membranes. The significant drop in signal intensity at energy levels below and above 0.12 joules demonstrates the limited optimal range for UV treatment.

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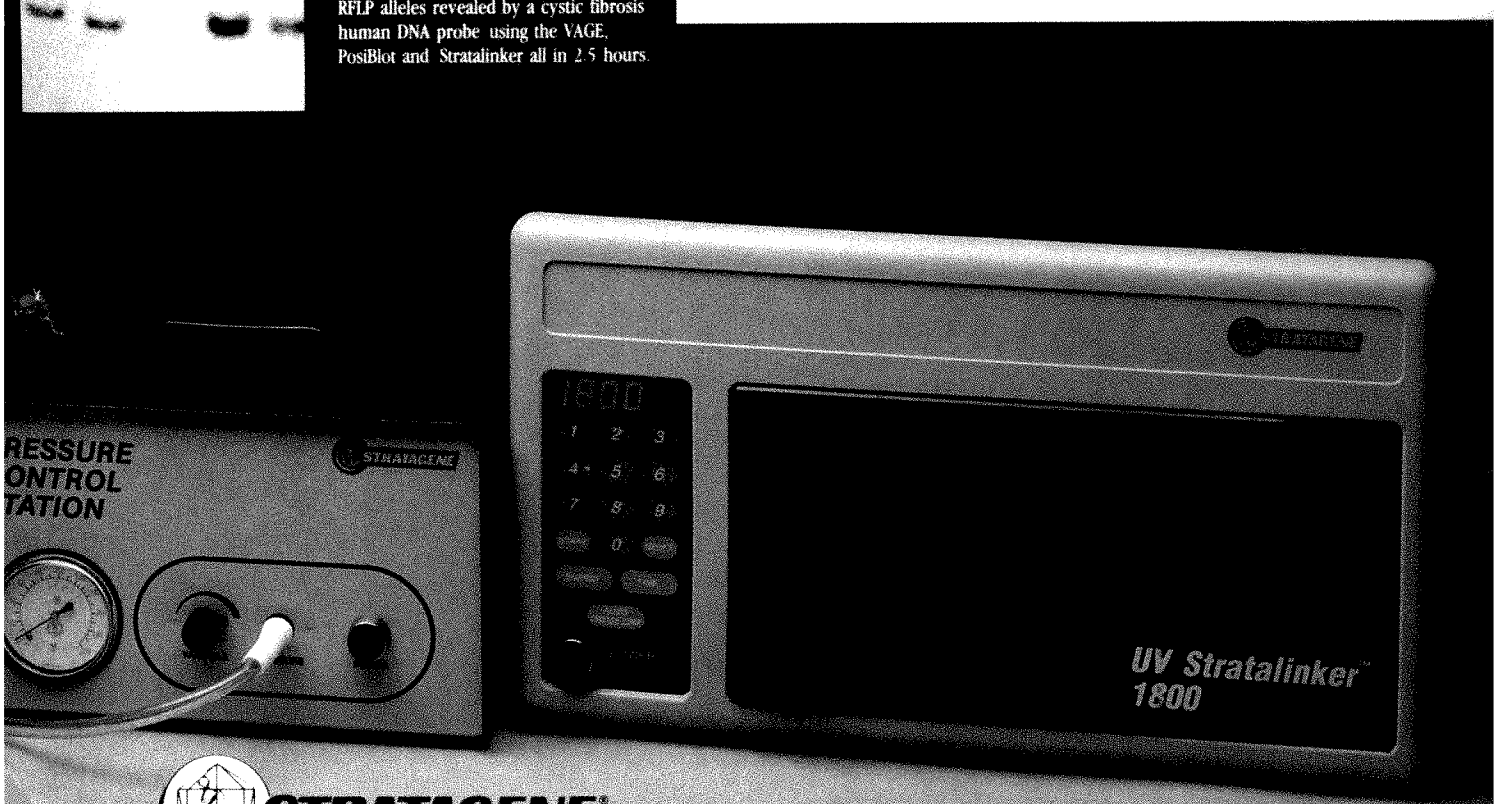

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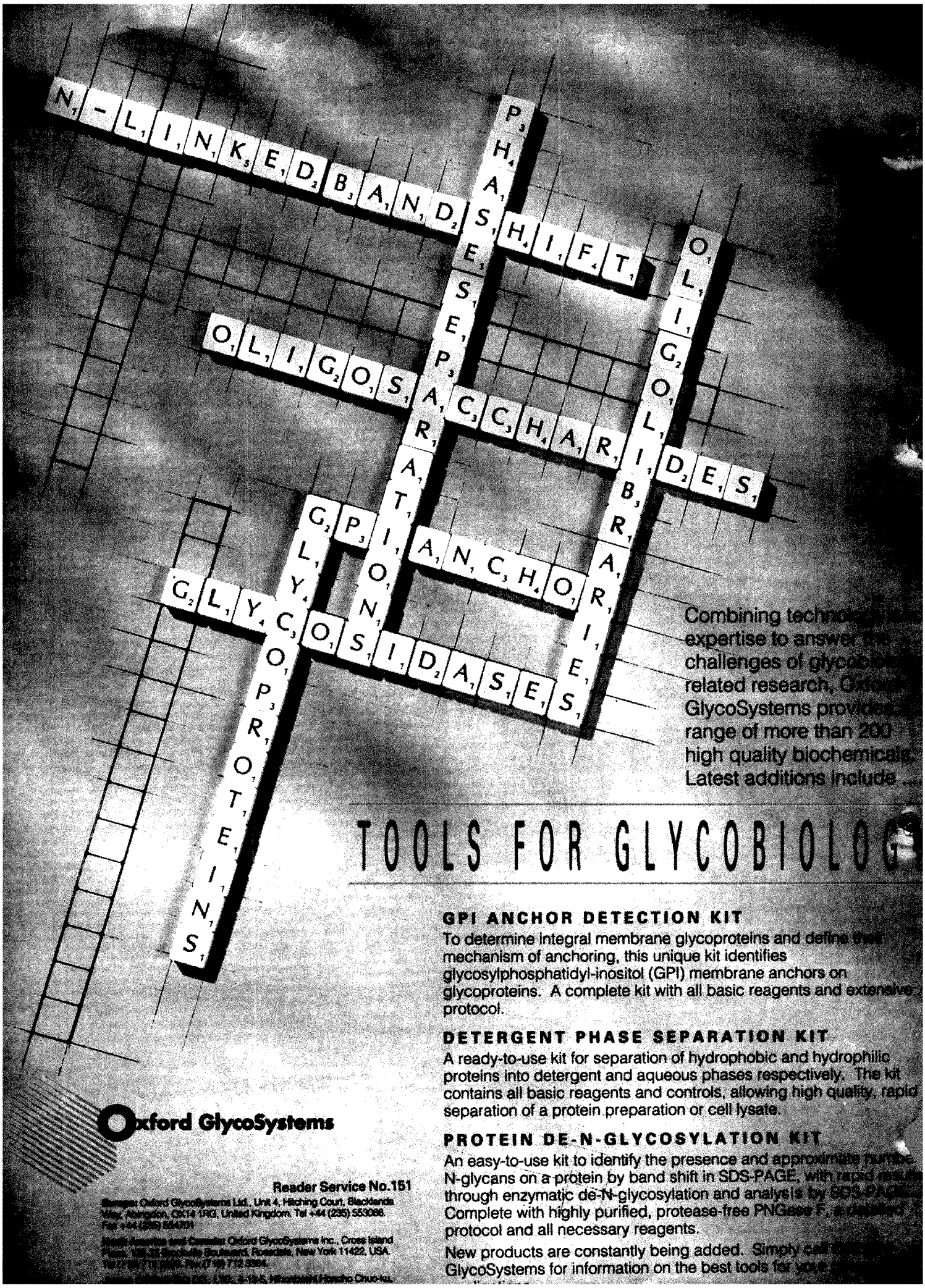

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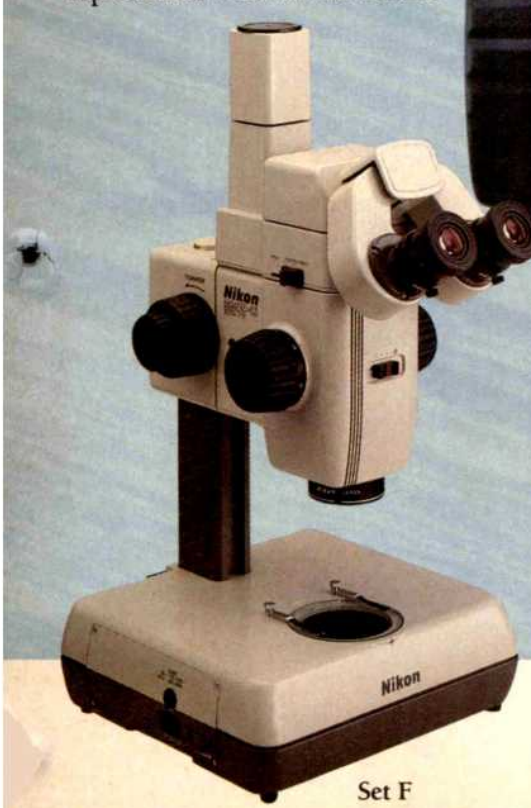

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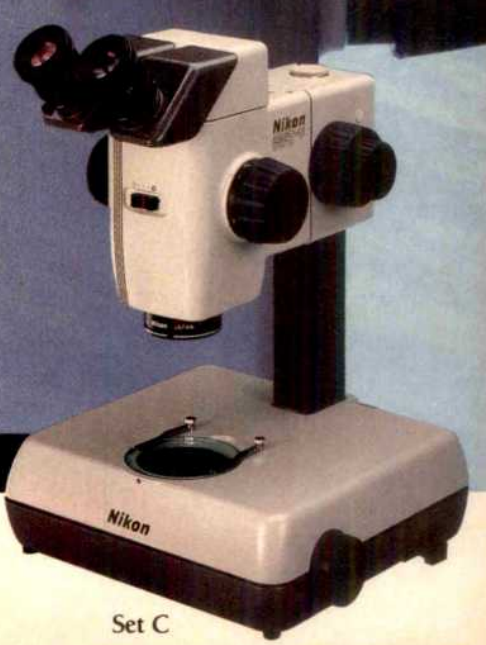

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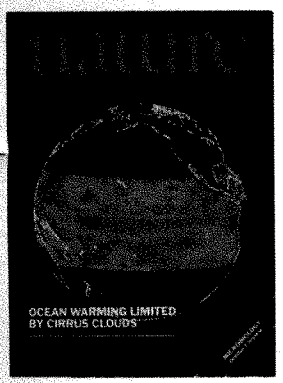

nature

2 May 1991

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◀ Is there a natural thermostat to prevent a runaway greenhouse effect? A 'super greenhouse effect' occurs during an El Niño climatic event, but the highly reflective clouds then produced shield the oceans from further heating. Cover: April 1987 (El Niño) minus April 1985 greenhouse effect. See pages 27 and 14.

THIS WEEK . . . THIS WEEK . . . THIS WEEK . . .

Sexual attractions

Although competition between males is the norm, there are many cases known where females compete for males. On page 58 Clutton-Brock and Vincent show that the sex theoretically able to produce more offspring is the one that actively competes for mating partners.

Digging deep

The mechanism of shallow earthquakes is well understood but deep-focus earthquakes remain mysterious. On page 50 Kawakatsu provides evidence that deep 'quakes', like shallow ones, occur primarily by shear slip on fault planes, rather than by spherically symmetric implosion.

Eleven-year glitch

The NASA Solar Maximum Mission satellite launched in 1980 has now monitored solar luminosity through virtually a complete 11-year solar cycle. Variations in luminosity seem broadly to coincide with solar activity in the form of sunspots and magnetic fluctuations, although excess irradiance in 1980 remains unexplained. Page 42.

Etched in the ice

Analysis of part of the harvest of Antarctic micrometeorites described in *Nature*'s 'Research in Antarctica' supplement (Vol. 350, page 300) is reported on page 44. An extraterrestrial origin is confirmed for much of the material, which may represent a new population of Solar System objects.

Lobster quadrille

Under the appropriate stimulus, neurons belonging to existing central pattern generating circuits in the lobster are recruited into a new circuit controlling swallowing-like behaviour. This glimpse of how a neural network dramatically reorganizes itself for behaviour sheds new light on central nervous system organization. Pages 60 and 18.

After unity

The funding of molecular biology in the newly unified Germany is discussed in this week's Commentary by Bruno Müller-Hill, who argues that teaching and research should be concentrated in the universities. Page 11.

Gene transfer

Identification of a gene in human herpesvirus-6 which encodes a polypeptide homologous to the product of the *rep* gene of human adeno-associated virus type 2 provides evidence of gene transfer between eukaryotic DNA viruses. Page 78.

Laser display

A novel mechanism for inducing alignment in nematic liquid crystals using polarized laser light is reported on page 49. The stable but reversible 'optical printing' that this process makes possible might have application in liquid-crystal displays, memories and micro-assembly. News and Views, page 15.

Diatomic iron

The idea that increasing atmospheric concentrations of carbon dioxide might be countered by the 'fertilization' of marine phytoplankton with iron salts depends on iron being the factor limiting plankton growth. So the finding that an oceanic species of diatom has a much lower iron requirement than a related estuarine species counts against this method of combating the greenhouse effect. Page 55.

Platelet synthesis

The stimulation of neutrophils with f-Met-Leu-Phe reveals that PtdIns(3,4,5)P_3 is produced *in vivo* by a different biosynthetic pathway than that thought to occur in blood platelets, in this case a route involving the enzyme PtdIns(4,5)P_2-3-OH-kinase. Page 33.

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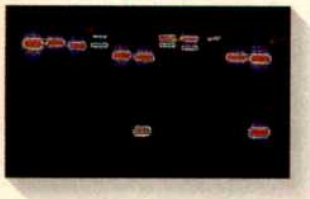

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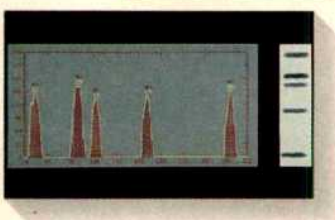

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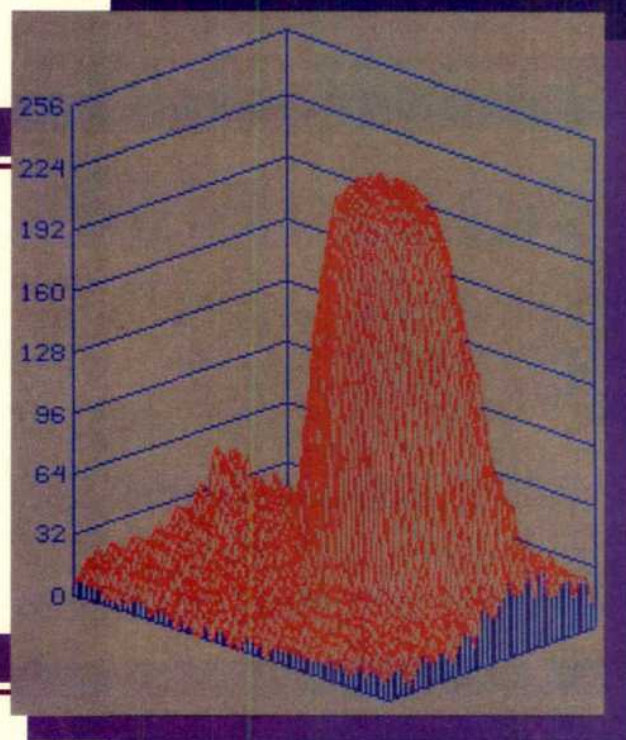

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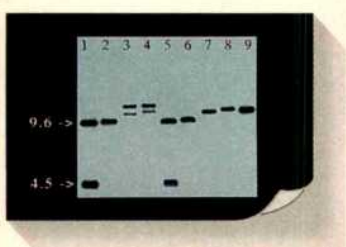

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2. Bauer, J.C., and Fox, J.S. (1991) *Strategies* **4**: 6-7

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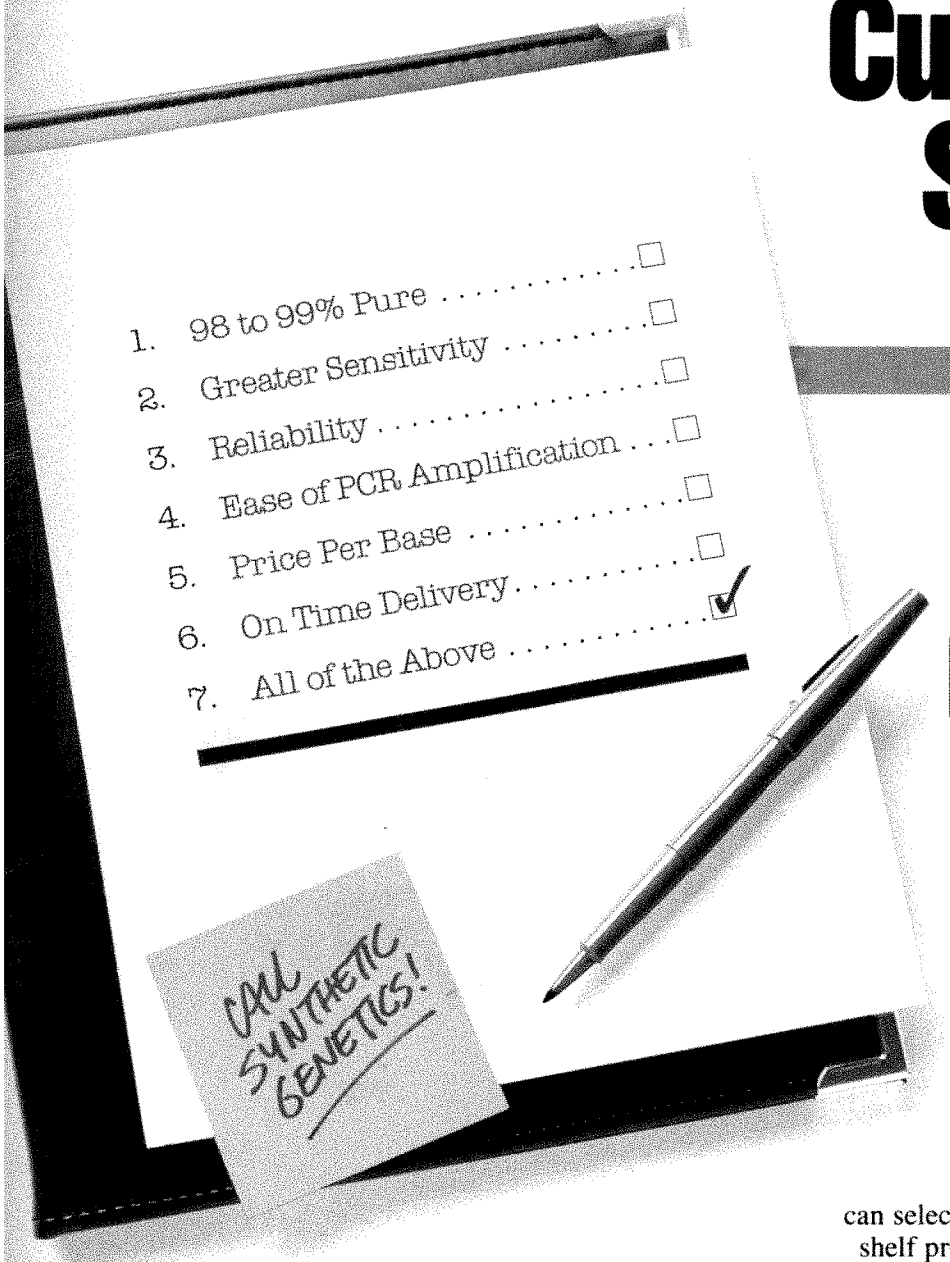

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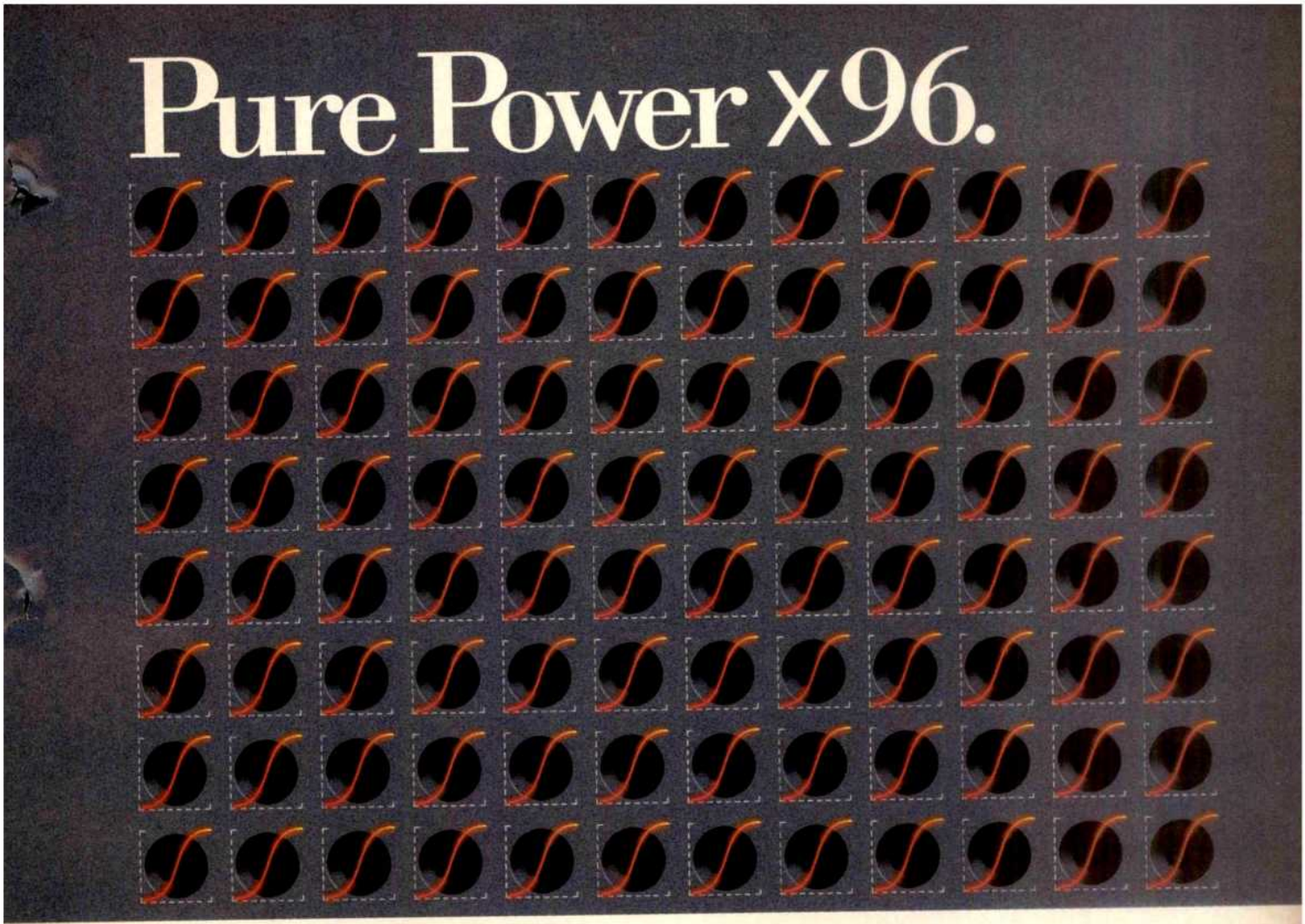

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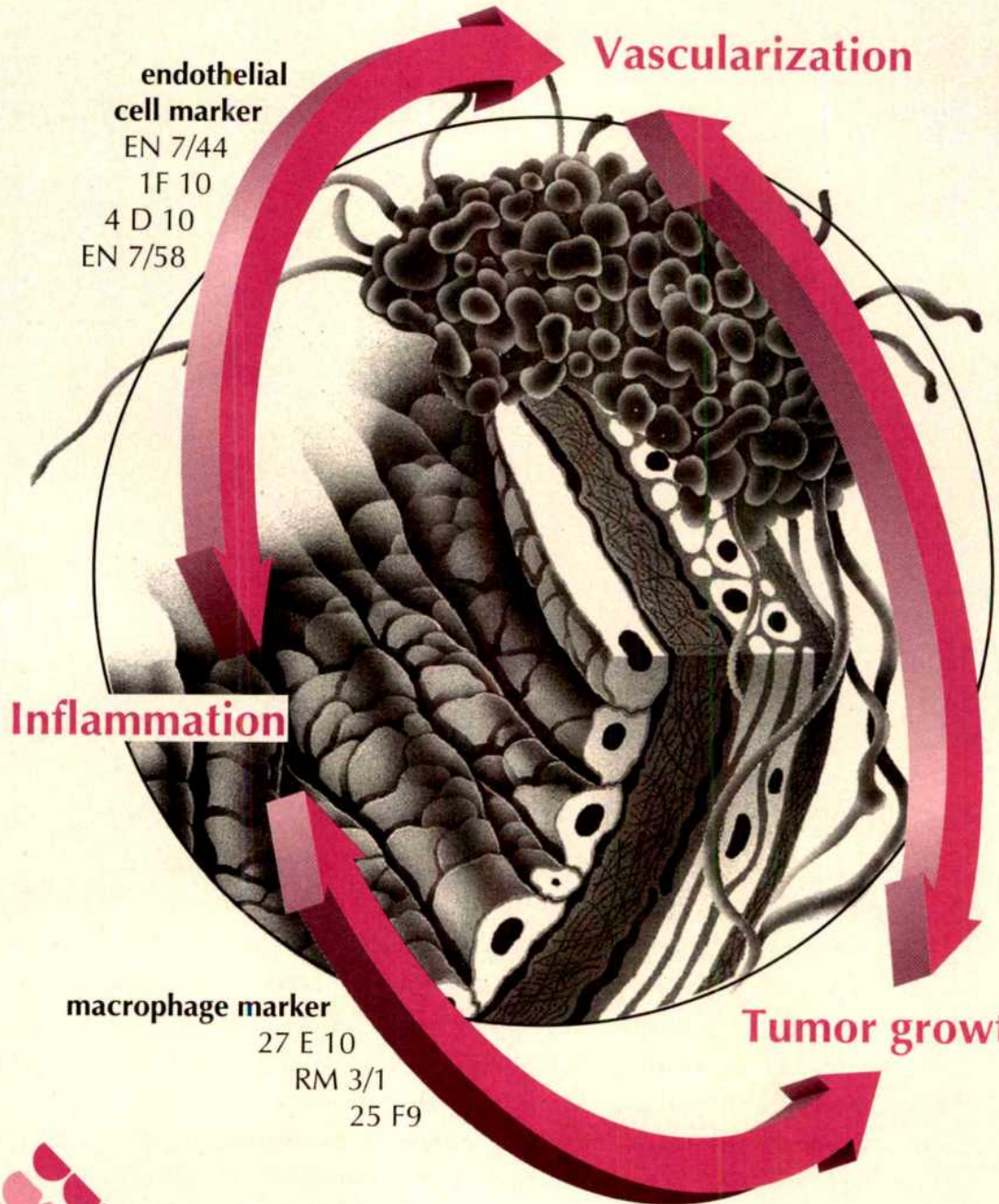

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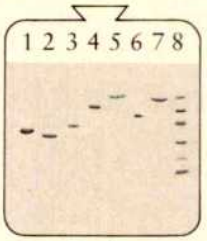

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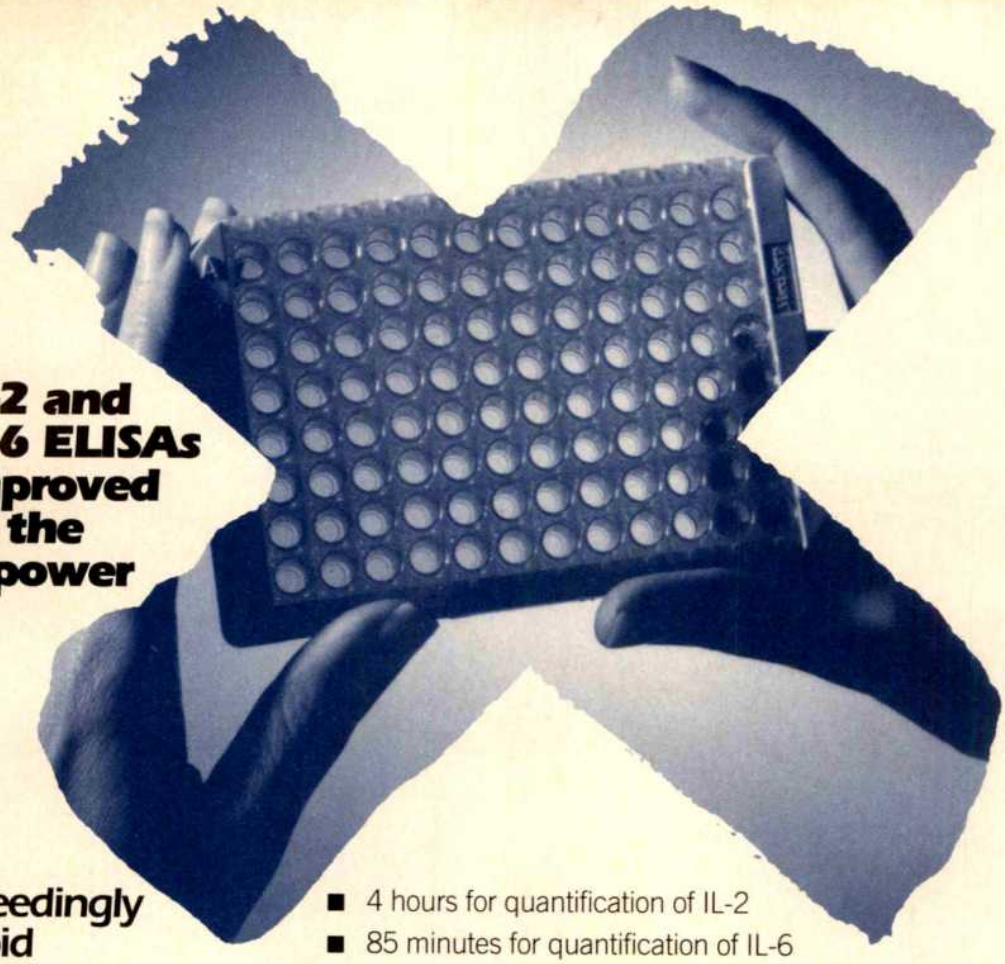

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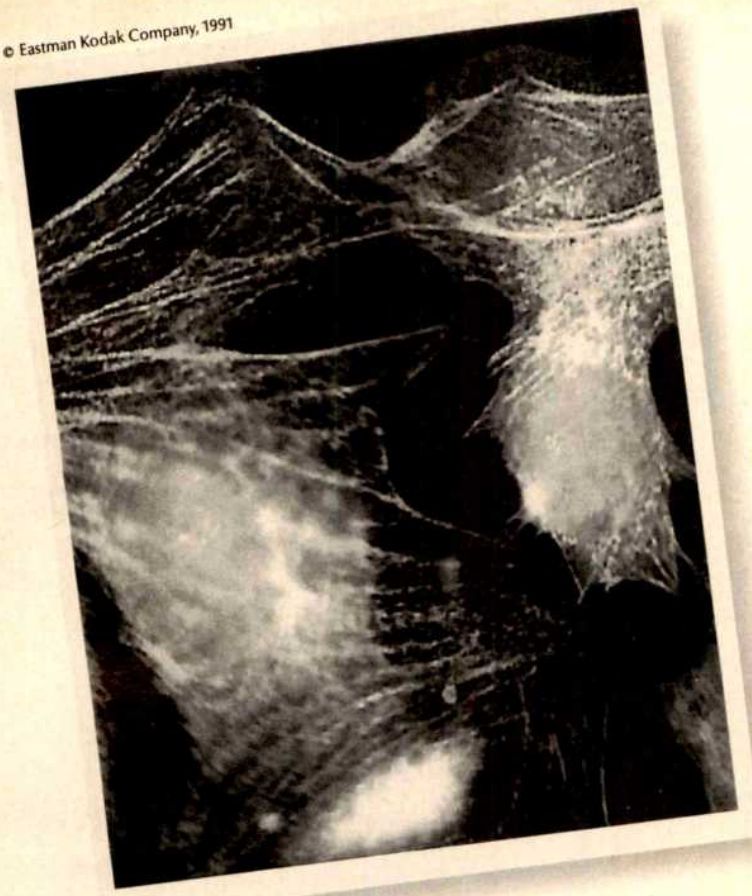

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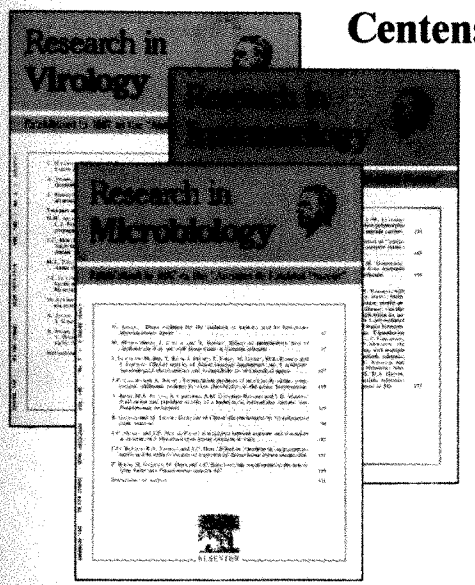

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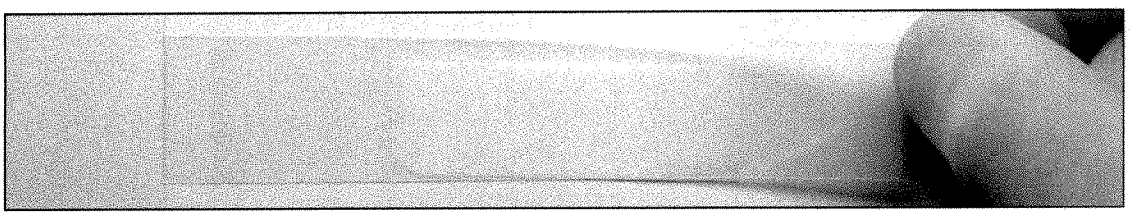

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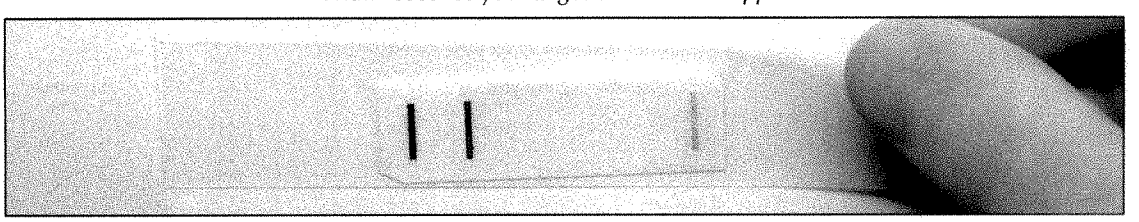

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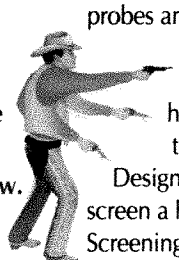

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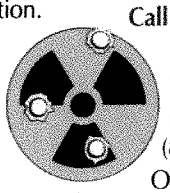

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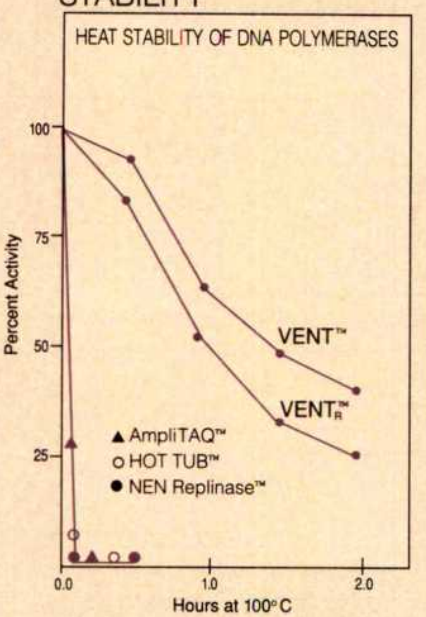

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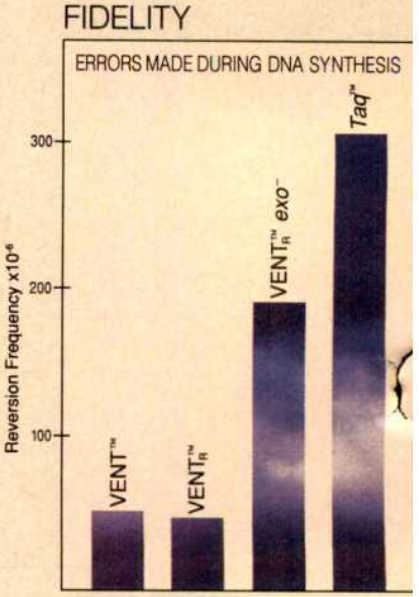

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Facing up to the Chernobyl accident

It is natural that the worst nuclear accident ever should have cast a long shadow, but last week's commemoration of the event and its likely repetition may have gone too far.

If the Soviet Union was not suffering such turmoil and if *glasnost* had not been dimmed by the events of the past few months, it would by now have been possible to piece together an account of what really happened at Chernobyl and in its aftermath five years ago (see page 4). But even now, there is no reason seriously to doubt the account given by the Soviet authorities six months after the accident, at the meeting organized in Vienna by the International Atomic Energy Agency. The cause of the accident was an ill-planned and unauthorized experiment carried through incompetently in circumstances in which malfunctioning nuclear reactors would not have been considered more remarkable than, say, trucks that had broken down.

The same slackness accounted for the failure to foresee the seriousness of the accident, or even to over-react to it, with the consequence that large numbers of people living nearby were needlessly exposed to radiation. The steps taken permanently to isolate the reactor were heroic, but in retrospect may have been characteristically hurried. The exposure to radiation of people living far from the site in the Ukraine, and especially in Byelorussia, has not been disclosed with the clarity its potential seriousness demands. The technical deficiences are those with which the Soviet Union is struggling, still ineffectually, in all industrial fields. The others are consequences of the Soviet difficulty, five years ago, of facing unpalatable truths.

Matters have since become worse in many ways, not better. Chernobyl has become an issue on which nationalist interests may seek to best the centre at Moscow, whence last week's mock trials and protest meetings, while the whole affair has become a symbol to people throughout the Soviet Union of the past mismanagement of their affairs. For those of us who live elsewhere, it might seem that the moral is that one should never buy a nuclear reactor from a Soviet organization, or rely on Soviet management for environmental protection, but there is more than that to say. First, the rest of the world needs to know exactly what has happened after Chernobyl so as the more confidently to face the decisions it will soon be making on alternative sources of power. Second, the Soviet Union's own reputation requires a proof that it has dealt humanely with all those affected by the accident.

The remedy is in *glasnost*, which means the technical publication of the details. What has happened to those evacuated from the surroundings of the site, what does cytology say about their exposure in the aftermath of the accident, where are they now and what arrangements have been made for their supervision and care? Tragic though the circumstances may be, information such as this bearing on the influence of small radiation doses on people's health will become with the passage of time an invaluable resource. The same is true of the records of those more heavily exposed to radiation at Chernobyl. The only sufficient memorial to those who died there is that it should be made public in exhaustive detail.

The consequences of Chernobyl outside the Soviet Union have been what the authorities first claimed — minimal. Contamination of reindeer in Northern Scandinavia has abated. A few spring lambs are still considered to be over-contaminated with caesium at two sites in the British Isles, but only because of the precipitate tenfold cut in European safety levels in the first few days after the accident, since when there have been five further years of mostly trouble-free operation at more than 200 reactors on the scale of that destroyed five years ago. The implications of that experience also need serious consideration. Nobody will again pretend that nuclear power is free from risk, but the past five years have also emphasized what has always been plain — that good management can reduce both the risks of accidents and their seriousness.

Different governments and their public utilities will rightly respond differently to these developments. Some who have always been sceptical of commercial nuclear power will remain so, at least for the time being. Some who were once sanguine may have become sceptical because of Chernobyl. But all of them, and also those wishing to push ahead with the development of nuclear power stations, will be helped enormously by more and better information. After five years, that is the best way of marking Chernobyl. □

Recovery by decree

Is President George Bush more alarmed by the threat of a recession than he has been telling?

The seven rich countries' finance ministers, in Washington last week-end for a meeting of the International Monetary Fund, were probably startled to be summoned by President George Bush to the White House and asked to reduce current interest rates in concert. It is as if the organizers of the annual meeting of the Federation of American Societies of Experimental Biology in Atlanta last week had been faced with a plea from City Hall also to use the occasion to halt the

growth of the world's population, arguably the most serious problem with roots in biology. In the event, the finance ministers appear to have heard out the president politely; they even issued a mildly approving statement. But there will be no concerted move in that direction.

Nor can there be. As Tolstoi might have put it, during economic growth, industrialized economies are much alike, but in recession they go separate ways, looking out for their own interests. On this occasion, for example, both Germany and Japan are more worried by the damage that would be done by inflation than by recession (the growing unemployment in eastern Germany notwithstanding), and are well within their rights to be so. But even if the finance ministers had done what the president had asked, the threat of a further deepening of the recession would not have vanished.

The theory is impeccable: if interest rates fall, people are more likely to borrow money to found new enterprises, so increasing employment and the output of goods and services. That is what happens when things are going well, so that interest rates are valuable economic regulators at times of growth. But when economies are shrinking, people are not tempted to invest in new enterprises by marginal reductions of the real interest rate (nominal interest rate minus the inflation rate), which now vary from 3 to 6 per cent a year in different countries; instead, they are more concerned that an investment that is unwise, perhaps because the market for what it will produce has disappeared, is a recipe for losing everything. The same people are still smarting from the ample illustrations during the recent unwinding of the 1980s boom of how apparently solid assets — investments in commercial banks, for example — can vanish into thin air.

So how, and when, will President Bush's wish be granted? The usual statement — by a return of "confidence" — is tautologous. The real question is that of what conditions must be satisfied before confidence returns. The passage of time, or forgetfulness, will help, but who can wait that long? In contrast with the early 1930s, the US administration has this time set its face against the protection of its domestic market; exporting to marginally more buoyant economies is a useful safety valve, but not a solution of every recessionary economy's problems. President Bush might more usefully have asked at the week-end whether a further deliberate liberalization of world trade would accomplish the trick he hopes for. That, and the encouragement of technical innovation, are the only remedies in sight. □

Shakespeare's school

There is yet another chance to reform British education, in which Shakespeare is only one component.

PRINCE Charles, the British Queen's heir, who delivered this year's Shakespeare Birthday lecture last week, regrets that the Bank of England has decided that Shakespeare's portrait will not always appear on the face of its £20 banknotes. The regret, no doubt, would have been well received by an audience of shakespeareans, but Prince Charles did not tell it that Shakespeare is to be replaced by Michael Faraday, another English genius. Would that have made a difference?

Most probably, not. The first part of the prince's address was a splendid evocation of Shakespeare, the second a comment on the state of British public education which has been widely regarded as politically controversial (but which is hardly so). The two themes were linked by the argument that Shakespeare's unique insight into the human condition was, by good fortune, written in English, for which reason English-speaking people and the British in particular have a unique component of their cultural heritage to cherish, to cultivate — and to teach in the schools. There is nothing controversial in that, or in the prince's assertion that culture should be a crucial part of general education. So much must be generally agreed.

What is most wrong with British school education outside Scotland (which has more liberal arrangements) is that it is not general education at all, but for many students an inadequate preparation for a life of specialism. It is commonplace that young people with an interest in science are required to commit themselves for, or perhaps against, when they are younger than sixteen; changing course later is difficult, often impossible. But the system is also hag-ridden by examinations, among which the dominant are the examinations (called A-levels) in which success is required for entry to universities and higher education. Only in the past few years has this system been broadened by the introduction (in this academic year) of less demanding courses that universities and polytechnics will accept. But even now, many students will be encouraged by their schools to stick with the traditional pattern, while the educational system is only now waking up to the need that courses in higher education should last for four years, not three. (The latest voice in support of that was a report from the Advisory Council on Science and Technology published earlier this week.) Can anybody wonder that the recruitment of young people into science and technology remains a British headache?

Both Prince Charles's proper interest and that of science and technology would be met if some British government (why not the present government?) were radically to transform and rationalize the system by requiring that there should be no one-to-one link between schools and higher education. Then schools could provide general educations and examinations that were proofs of competence. Universities and polytechnics might have to devise other ways of fitting would-be students to the courses they offer, or switch to open access as others do. But there are two obstacles. One is the pecularly British conceit that it is feasible to measure attainment so accurately that a young person's educational destiny can be accurately foretold (which rather defeats the objectives of education).

The other, clear from Prince Charles's speech last week, is the contrast between culture and other kinds of intellectual work, in which the former is preferred. Although the Bank of England's decision to replace Shakespeare by Faraday has been compelled the need to redesign all banknotes to be the same size, the switch may help dispel the entrenched conceit. □

US state universities face massive cuts

■ Recession brings layoffs, programme closures
■ Long-term effect on research still unclear

Washington

A RASH of state budget crises is undermining a decade of US academic growth, as more than half of the university systems in the 50 states now face serious cutbacks and deficits.

Over the past few months, 26 state university systems have been forced to cut their budgets for the 1991 fiscal year — money that had already been committed to salaries and current programmes. Next year, matters look even worse. At least eight state governments are planning to cut their university systems below this year's level — as much as 20 per cent below, in the case of the University of Massachusetts system. Another 16 states do not expect to keep up with inflation in their higher education funding, even in the face of larger numbers of students entering the university systems.

Two year ago, state universities were riding on the crest of a wave of increased enrolment, new programmes and construction that came with the academic boom years of the 1980s, a period in which the state university systems grew on average by 35 per cent.

In 1989, more than three-quarters of all US higher education students attended state colleges and universities.

But then the recession struck, leaving many universities back at 1980s-level funding despite increased enrolments and classes. Most systems have responded by cutting administrative and support staff, postponing construction and equipment purchases, raising tuition and student fees, delaying salary increases, offering early retirement to tenured faculty and eliminating some departments and courses.

It is hard to know what effect the current crisis will have on long-term academic quality. Most university research programmes are supported on federal grants and are largely sheltered from the fiscal storm. Of the programmes that are being cut, many are in fringe or shrinking fields, such as home economics or traditional agriculture. In times of plenty, universities protect such programmes to avoid faculty and student protest. But in lean times, when the need is clear, it is easier to make tough decisions without triggering student demonstrations.

If restructuring trims university programmes back to a lean core of key disciplines, that may not necessarily be a bad thing, despite the loss of academic diversity. "We can't be all things to all people anymore," says Donald Langenberg, chancellor of the University of Maryland. "We've got to focus on what we can do best. Now we can make the cuts we should have made all along."

Like universities elsewhere across the country, University of Maryland officials are planning to eliminate faculty and staff positions, close programmes and departments, and increase class sizes. A 'strategic redeployment' is expected to eliminate two colleges — Human Ecology (what used to be known as Home Economics) and Library & Information Service — and eight other programmes, including nuclear engineering and hearing and speech sciences.

University associations blame the trouble on the nation's general economic malaise, coupled with the Reagan Administration's eight-year policy of transferring responsibility for federal social programmes to the states. Just one of those transferred programmes — Medicaid, health-care support for the poor — will cost the states some $67,000 million in 1995, more than twice what they spent last year. Facing political pressure against increased taxes, the states were forced to dip into general funds, out of which the university systems are funded.

"Higher education is a big piece of the pie and it's discretionary. That makes it easy to cut," says University of Maryland spokesman John Lippencott. Maryland's higher education budget consumes 10 per cent of the total state budget — a tempting target, and one that did not go unnoticed. Last month, Maryland state legislators cut $67 million — 12 per cent — from the state university budget.

Cuts at other state university systems include:

■ California: In the California State University system, which faces a $402 million cut from its 1992 request, officials are planning a 20 per cent student fee increase and layoffs of 864 staff, instructors and part-time faculty. They intend to lose 420 faculty positions by offering early retirement. Chancellor Ellis McCune told the state assembly that in his 30 years within the CSU system, "I have never seen a budget that promises to be as devastating."

Legislators have cut $295 million from the 1992 request of the University of California system. In response, UC officials are planning to cut 1,000 staff and defer faculty salary increases for six months. They expect to reduce enrolment by 5,500 students, resulting in the elimination of 360 faculty, 100 teaching assistants and 300 support staff. Officials also plan to cut $20 million worth of programmes that are yet to be named. A one-time 'Golden Handshake' early-retirement offer earlier this year was accepted by 3,000 staff, about half of those eligible.

■ Massachusetts: The University of Massachusetts system faces a $115 million cut from its 1992 request. Governor William Weld plans to close as many as five colleges, eliminate 1,000 faculty positions and increase tuition by one-third. The university plans to kill its Community Health Education Program and part of its School of Education. Spokeswoman Kay Scanlan says, "Before the legislature started on the new budget, we had asked all our departments to turn in budgets reflecting a 7 per cent decrease from last year. Now we don't even know what to ask for. Forget keeping up with inflation."

■ New York: The State University of New York (SUNY) system request for 1992 has been cut by $60 million, forcing a 30 per cent increase in tuition fees and 2,300 personnel cuts. At the City University of New York (CUNY) system, a $92 million cut has resulted in doubled student fees and class closures at six of 21 campuses. CUNY may lose 810 faculty and staff positions at a time when enrolment is up 15 per cent. W. Ann Reynolds, chancellor of CUNY described the predicament as 'the toughest fiscal situation since the late 1970s.'

■ New Jersey: State universities have weathered $72.5 million in cuts over the past three years. Rutgers University plans to cut programmes, 200 staff and 160 teaching positions to make up its largest ever $38 million reduction this year. The New Jersey Agricultural Experiment Station, located at Rutgers, will lose $6.6 million.

■ Oregon: A state-wide property tax cut has meant an $86 million loss to the university system in an otherwise healthy Oregon economic climate. The reduction will mean the elimination of 700 faculty and staff positions and some $50 million in programme cuts.

Portland State's Health and Human Performance School will be closed and its Centre for Public Health Studies is to be absorbed by another department. The University of Oregon will close its College of Human Development and Performance, and mathematics, English, science and speech teacher education programmes.

■ Virginia: The University of Virginia system, which faces a $46 million cut, intends to let attrition eliminate 153 faculty jobs, and to impose a 2 per cent salary cut on all remaining faculty.

Christopher Anderson

World researchers to take a closer look at Chernobyl

Munich

FIVE years after the nuclear disaster at Chernobyl, the world may finally get a closer look at the health effects of the radiation released by that accident.

Until recently, the Soviet Union has not been cooperative about releasing details of the calamity, but now, in conjunction with a programme of the World Health Organization (WHO), it will apparently open its doors to health researchers from the rest of the world.

On 6 May, the World Health Assembly, which is WHO's parliament, is expected to vote to lend WHO support to an international research programme to study the effects of Chernobyl. The programme, which began last year as a Soviet pilot project, will allow international researchers to gain unprecedented access to the Soviet population in order to carry out epidemiological and other studies and to track the effects of the accident on the incidence of cancer and other diseases.

A year ago, the Soviet Union began to set up a research centre at Obninsk, 100 km southwest of Moscow, to coordinate research on the effects of the accident. The Obninsk site was chosen because it already houses 15 research institutes of the Soviet Academy of Sciences that deal with various aspects of radiation and nuclear science. At the same time, Soviet officials also began to set up satellite research centres nearer to Chernobyl itself in each of the three republics most strongly affected by the accident, the Ukraine, Byelorussia and the Russian Federation.

Earlier this year, Japan became the first WHO member state to volunteer assistance for the programme, pledging $20 million to improve the facilities at Obninsk. The pledge came in response to a letter to the Japanese government written by Hiroshi Nakajima, the Japanese director-general of WHO, which is based in Geneva.

And now that the World Health Assembly has given its stamp of approval to the programme, WHO officials say that other members of the organization (which has 166 member states) are more likely to follow the Japanese lead and make donations. WHO itself has offered to play a coordinating role for international cooperation in the Chernobyl effects study programme, but it is not in a position to devote any of its own budget to the programme.

Long-term support for the programme is an absolute necessity, WHO officials emphasize, because many of the cancer cases will not emerge for another 5–15 years or more.

The programme will attempt to track the progress of several types of cancer, the incidence of which is expected to rise because of exposure to radiation. The first cancers and other abnormalities to emerge in the next few years are expected to be leukaemias, followed by thyroid disorders after a 15- to 20-year latency period.

Another focus of the programme will be to assess the psychological and psychosocial effects of the disaster. Inspection teams from WHO and the Vienna-based International Atomic Energy Agency have reported that the largely rural local population blamed a variety of illnesses, especially among children, on radiation, even when this was clearly not the cause. The reported increase in children's illness, and in high blood pressure, anaemia and pulmonary disorders in the adult population are thought to be due more to feelings of depression and helplessness among the population than to the effects of radiation.

One potential stumbling-block for the programme is that it may not be able to determine how many cancers are due to the Chernobyl accident because Western experts say there were very few data available from the region before 1986 to use as a baseline for the study.

Steven Dickman

Five-year toll: 10,000 dead from Chernobyl?

London

How many people have really died because of radiation released by the Chernobyl accident? A Soviet scientist has reopened that question with his claim that the Soviet Union has covered up thousands of deaths, but other scientists remain sceptical.

Vladimir Chernousenko, a member of the clean-up team sent into the Chernobyl area after the accident, claims that between 7,000 and 10,000 of the 600,000 so-called 'liquidators' brought in to clean up after the accident have died because of exposure to radiation. The official death toll is 31. The claim, made by Chernousenko in a British television documentary programme, broadcast by Thames Television, has been dismissed by the Soviet Ministry for Nuclear Power and Industry, and questioned by Western experts who have studied the consequences of the Chernobyl accident.

John Gittus of the British Nuclear Forum, who led the UK Atomic Energy Authority team studying the consequences of Chernobyl, says that non-acute health problems caused by radiation should be impossible to detect only five years after the accident. For solid cancers, studies of radiation-exposed populations, such as the Hiroshima and Nagasaki bomb survivors, show a ten-year latency period before excess cases appear. The UK Atomic Energy Authority estimates that Chernobyl will eventually cause 10,000 excess cancer deaths in the Soviet Union. This represents only 0.03 per cent of the natural Soviet cancer rate.

Evelyn Sokolowski, from the Swedish Nuclear Training and Safety Centre, says that the death toll given by Chernousenko is consistent with the natural mortality rate in the Soviet Union among a population of 600,000 averaging 29 years of age.

In an apparent attempt to discredit the cancer claims, Soviet authorities initially said that Chernousenko had no scientific credentials to discuss the effects of Chernobyl. In a biography distributed by the documentary's producers, Chernousenko had been described as the scientific director within the 30-km exclusion zone that surrounds Chernobyl, and deputy chairman of the Ukrainian Academy of Sciences Commission responsible for rectifying the consequences of the accident. The Soviet authorities denied that Chernousenko was even a scientist.

Those authorities have now issued a second statement, quoting Victor Bar'jakhtar, chairman of the Ukrainian Academy Commission on Chernobyl. This acknowledges that Chernousenko is a senior scientist at the Ukrainian Academy Institute of Theoretical Physics and a consultant to the Commission on Chernobyl, but says he is not the commission's vice-chairman.

Despite the general rejection of Chernousenko's fatality claims, some scientists who have visited the Chernobyl site are concerned about the safety of the Soviet scientists still working at Chernobyl — some are even working inside the concrete 'sarcophagus' built to enclose the shattered reactor.

Brian East, head of health physics at the Scottish Universities' Research and Reactor Centre, says that Soviet physicists are working in primitive plastic oversuits in an area likely to be rich in plutonium, and seem to have no equipment to measure surface contamination with alpha particles; in the United Kingdom, workers entering areas where plutonium is being manipulated wear sealed suits with a positive pressure.

A more complete analysis of the health and environmental effects of Chernobyl will be released later this month by the International Atomic Energy Agency, which has co-ordinated a study involving a number of United Nations organizations.

Peter Aldhous

JAPAN

Pollution on the upswing

Tokyo

JAPANESE industry has set an example for the world by reducing its energy consumption and carbon dioxide emissions through energy-efficient technology and production processes. But Japan's 120 million citizens and the nation's trucking companies are rapidly undoing all this hard work. According to the latest annual white paper (policy document) on the environment, released last week by the Environment Agency, Japan's consumer and transport sectors are steadily increasing their consumption of energy and emission of pollutants — cancelling out the environmental gains made by Japanese industry.

Although the small and comparatively young Environment Agency has little political clout in the Japanese government, it has become increasingly bold in its pronouncements, reacting to growing concern about the global environment. And its recent white papers have attracted worldwide as well as national attention.

The white paper, in what the agency claims is a first for any country in the world, breaks down carbon dioxide emissions in Japan into four sectors: industry, private households, transport and energy conversion and electric power transmission losses. The breakdown provides a revealing insight into where Japan's carbon dioxide problems lie.

Analysis of emissions over the past 25 years reveals that carbon dioxide discharges from industry fell significantly after the 1973 oil crisis, as industry rallied to improve its energy efficiency. Industry's share of total emissions has accordingly dropped from 60.2 per cent in 1973 to 49.5 per cent in 1989. And this was achieved against a background of a rising gross national product and industrial production (except for a brief period of negative growth immediately after the oil crisis).

On the other hand, consumption of energy and carbon dioxide emissions in private households have continued to rise steadily. Households now account for 23.2 per cent of all emissions, compared with 18 per cent in 1973, and their share of electricity use has also expanded dramatically as more and more Japanese homes have installed air-conditioning units and other electrical appliances.

Similarly, the share of emissions from the transport sector climbed from 13 per cent in 1973 to nearly 20 per cent today. According to the white paper, the biggest culprits are the small- to medium-sized companies that operate the thousands of diesel trucks that now jam Japan's narrow roads. The trucks not only emit carbon dioxide but also contribute much of Japan's nitrogen oxide (NO_x) pollution.

Paradoxically, Japan has some of the strictest regulations in the world for control of emissions from cars. The regulations were introduced because of severe urban pollution problems in the 1960s and early 1970s, and, as a result, NO_x pollution in Japan's cities decreased steadily from a peak in the mid-1970s until the mid-1980s. But the white paper shows that since 1985, NO_x pollution has been on the rise again, and the major source of this pollution is diesel trucks.

Emission controls for diesel engines are not so strict as for gasoline-driven vehicles and, because diesel fuel is considerably cheaper than gasoline, there has been a boom in the use of diesel trucks among small trucking companies, which have expanded their operations dramatically in recent years. The boom in the trucking business has been caused by increased demand for private parcel delivery services and by the general expansion of consumption of products in Japan's increasingly affluent society.

Ten years ago, the millions of small convenience stores and groceries that line the streets of Japanese cities managed with only one or two deliveries by truck per day. But now, as manufacturers compete ferociously to grab a share of the market, dozens of deliveries in a day are not uncommon. One example, cited in television reports last week, is the current 'beer war', where beer manufacturers have flooded the market with 174 marginally different brands of lager beer — all of which need transporting to retail outlets.

The problem is compounded by Japan's grossly inadequate road system. Tokyo has a network of toll-charge 'expressways' built in the 1960s. But these major arteries are only two lanes wide and at some key junctions they narrow to one lane. Traffic jams that extend for miles are an everyday event. And the jams are getting worse every year as more trucks and cars flood the roads.

Kei Takeuchi, an economist at Tokyo University's Research Center for Advanced Science and Technology, who leads a team of researchers trying to find social and economic solutions to global environment problems, blames the Japanese government for much of the problem. It is all very well for the Japanese automobile industry to offer leading-edge emission controls and fuel-efficient cars, he says, but until the government takes the initiative to solve Tokyo's traffic jams, no amount of technology will ease the city's growing pollution problems.

A few months ago, the Tokyo metropolitan government tried to improve the situation by asking transport companies to refrain from using their vehicles on Wednesdays. But only 5 per cent of 5,500 companies complied.

Toshiro Kojima, director of the planning and research division of the Environment Agency, says the agency hopes that the price differential between diesel and gasoline fuel will be eliminated and tax incentives will be introduced to encourage transport companies to stop using diesel trucks. But because the trucks typically cost about ¥10 million ($70,000) each and last for years, he admits that a rapid switchover is unlikely.

David Swinbanks

ENVIRONMENT

One Westerner gets it RITE in Japan

Tokyo

LAST week, when the Japanese Research Institute of Innovative Technology for the Earth (RITE) announced the 12 winners of grants for research on the global environment, 11 were Japanese scientists, even though the programme was in theory open to researchers of all nations. The result is not too surprising, however, given the way in which the grants were publicized outside Japan — that is to say, not at all.

The episode illustrates how the Japanese sometimes send conflicting signals to the rest of the world — in this case, seemingly asking for outside participation but discouraging it at the same time.

RITE was established last year by the Ministry of International Trade and Industry (MITI) to develop 'environment-friendly' technology (see *Nature* 350, 266; 1991). As part of its task, it offered the 12 grants, which range in value from ¥3 million to ¥7 million ($22,000–$50,000) a year. MITI decided that the institute should open up the programme to non-Japanese as part of a policy to internationalize MITI's research projects.

But unfortunately for non-Japanese scientists, the New Energy and Industrial Technology Development Organization (NEDO), in conjunction with RITE, is responsible for funding and publicizing the grants. NEDO is a semi-government organization that runs most of MITI's projects, including RITE, and it does not seem to share the ministry's enthusiasm for internationalizing the projects.

For the RITE programme, English literature describing the grants was sent to foreign embassies in Tokyo, but the only other announcements appeared in Japanese publications and were written, of course, in Japanese.

Very few non-Japanese scientists noticed the announcements for the new programme before the deadline in late January, and only one will receive an award. S. C. Jarvis of the UK Agricultural and Food Research Council's Institute of Grassland and Environmental Research will get ¥6 million to study methods to enhance the capacity of agricultural soils to provide sinks for methane.

David Swinbanks

A global experiment in technology transfer

Irvine, California

In 1987, after a year of delicate and sometimes acrimonious debate, 31 nations signed the Montreal Protocol, agreeing to phase out their use of chlorofluorocarbons (CFCs) in order to save the Earth's ozone layer. Last summer, after more tricky negotiations, the developed nations even offered to pay part of the cost for the less developed countries. That, however, was just the easy part.

Now, countries around the world are struggling to determine how they will keep their promise to replace the valuable and ubiquitous CFCs. Researchers and engineers are trying to develop refrigeration systems and insulating foams that do not use CFCs. Politicians are debating the trade-offs among cost, convenience, energy efficiency and environmental damage that will have to be made with the substitutes. But perhaps the biggest challenge will be transferring the technology for CFC replacements from developed countries, where it is being devised, to developing countries.

Last week, the US National Academy of Engineering sponsored an international conference to review progress in developing CFC replacement technology and to map out strategies to transfer that technology to developing countries. As was clear by listening to the delegates from two dozen nations around the world, technology transfer is still more an ideal than a reality.

Yet the success of this technology transfer will determine more than just the final score in the battle against ozone depletion. In many ways, the fight for the ozone layer is a trial run for solving more difficult global environmental crises that may lie in the future. As conference participant Anthony Vogelsberg, environmental manager of Du Pont's fluorochemicals division, put it, "If you can't deal with this effectively, then how are you going to deal with global warming or other problems that come up?"

CFCs have a wide variety of uses, including refrigerants in refrigerators and freezers as well as in building and automobile air-conditioning systems; 'blowing agents' for making insulating and other types of foam; cleaning solvents used in the manufacture of computer chips; and the propellant in aerosol sprays. The chemicals, which consist of one, two or three carbon atoms with chlorine and fluorine atoms attached, are particularly stable, which allows them to make their way into the stratosphere, where they break down and release chlorine atoms, which destroy ozone.

Even before the Montreal Protocol, several of the world's major chemical companies were working to find chemicals that can replace CFCs, and it now appears that the replacement of CFCs will go smoothly in the developed world. It is the developing countries that will be the problem. Since the manufacture of CFCs is relatively 'low tech', many developing countries have their own manufacturing plants, mostly to supply refrigeration needs. They will have to give these up and — at first, anyway — be dependent on the large multinational companies which have the expertise and the capital to set up the plants for CFC substitutes.

The changeover will not be as simple as having the developing countries give up their CFC production and move to the replacement chemicals. Almost all of the equipment that uses CFCs — refrigerators and air conditioners, foam-making machinery, cleaning equipment in semiconductor-manufacturing plants and so on — will have to be replaced or retrofitted. Somehow, engineers and technicians around the world will have to be instructed in the design, manufacture and operation of a whole new generation of equipment.

This will demand technology transfer on an unprecedented scale. The participants at the conference reviewed a number of obstacles that they have encountered or expect to encounter.

■ The companies that are developing the CFC replacement technology wish to protect their patents and intellectual property rights — and make a profit — but developing countries want the technology as cheaply and with as few strings attached as possible. Originally, countries such as India had asked that the new technology be given to them free; however, the technology belongs not to governments but to corporations, which do not give away their assets. Indeed, companies such as Du Pont would prefer not even to sell the technology itself. Du Pont's Vogelsberg says the developing countries should "either buy from world-class plants or, if they want local manufacture, should have joint ventures."

The spectre of the Bhopal disaster at a Union Carbide plant in India is never far from anyone's mind in such discussions, and Vogelsberg says Du Pont would be unwilling to operate a high-technology CFC replacement plant in a less developed country unless the company had a controlling interest in order to guarantee the safety of the plants.

In countries such as India, where the government restricts foreign ownership, the multinationals are likely grudgingly to licence their technology, but that brings up another problem. The developing countries will ask the multilateral fund to pay their licensing fees, but $160 million will go only so far. At some point, the corporations can expect to be pressed to lower their fees on CRC replacement technology.

Furthermore, the corporations will be exceptionally cautious about selling their latest and best technology to countries such as India and China, both of which have reputations for not being completely respectful of intellectual property rights.

■ Many developing countries do not have the capital to finance the changeover to a totally new technology. A multilateral fund established last year by the signatories of the Montreal Protocol will pay only the extra costs associated with replacement technology, not the entire cost of new facilities. If, for example, a developing country wishes to build a refrigerator plant to take the place of an existing facility that makes CFC-based refrigerators, it must pay what a new CFC facility would have cost; the fund makes up the difference. But the developing nation must still invest a large amount of capital in the new plant — and swallow the costs of shutting down the old one.

One solution is for developing countries to invite multinational corporations to build new plants inside their borders in joint ventures, but that can run into the same Catch-22 as above, with both parties wanting control of the joint venture.

■ In countries such as Brazil, which has a history of successful joint ventures with multinational corporations, the problems centre instead on the lack of a government framework for technology transfer. Brazilian industry has already started to plan for a CFC-free future by forming a formal study group to prepare for the change over to new technology, said Alberto Carrizo, director of the engineering, research and development and quality division of Climax Industries in Brazil. The companies are interested in export business and recognize that they cannot afford to fall behind their competitors in the developed countries, he said.

But the Brazilian government has not moved as fast. "Nothing is happening in the government," said Suely Carvalho of the department of experimental physics at the University of San Paulo.

"We can have access to an international fund of over $100 million, but we do not have the institutional channels." Brazil needs to establish procedures for industries to get access to the multilateral fund, Carvalho said, as well as to put together a central clearing-house of information about CFC replacements.

Eventually, Brazil will want to develop an indigenous capability in this new industry, she said, and for that, "we need an integrated effort among society, research centers, industry and government."

■ The physical task of training workers and technicians in developing countries in the new technology seems overwhelming. Even in countries that import the replacement CFCs, the indigenous industries will need to learn to make new equipment that works on the new chemicals. Refrigerators and air conditioners will have to be redesigned, for instance. And because the technological infrastructure of developing countries is less sophisticated than that of the United States, Europe or Japan, the developing nations generally will have to redesign the technol-

ogy to match their own needs and capabilities.

So far, one success story in the effort to replace CFCs seems to be Mexico. There, according to Jorge Corona, the director of ecological studies for the National Chamber of Industrial Transformation, the government took the lead in deciding to phase out CFCs as quickly as developed countries and industry quickly jumped on board. With the coming free trade pact with the United States, Corona said, "it would be foolish to be 10 years behind our trading partners in developing the replacement technology."

Mexico is phasing out CFC aerosols faster than Japan, and it has cut its use of CFCs in foam manufacture by half, said Geno Nardini of Valvulas de Precision. It is still waiting for adequate substitutes for refrigeration and air conditioning uses, he said, but "local companies are preparing themselves so that when they get the okay from the US and Europe, they're ready to go."

Mexican industry has an advantage over that of many developing countries because of close ties with some of the multinational corporations that will be developing the replacement technology, and it intends to use those contacts for "seed help", Nardini said. But it plans to adapt the technology for its own uses and then help other developing countries to make the changeover from CFCs. "Once technology is adapted to one developing country, it is much easier to transfer to another country at the same stage of development," Corona said.

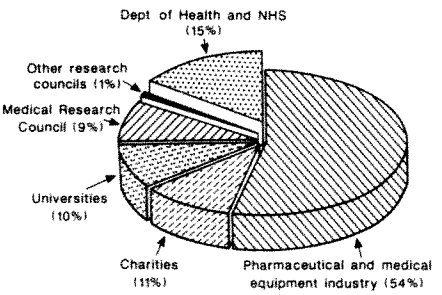
Like the other delegates at the meeting, Corona and Nardini recognize the importance of establishing the CFC replacement campaign as a prototype for the future. "This has helped the whole world study the mechanisms for fighting this sort of problem," Corona said. "If we had not had the experience we're having now dealing with CFCs, I don't think the world would be ready for dealing with global warming." **Robert Pool**

HEALTH RESEARCH

NHS launches first strategy

London

MORE than 40 years after its creation, the UK National Health Service (NHS) last week announced its first-ever comprehensive research strategy. Michael Peckham, director of research and development for the NHS since January, says the aim is to "pervade the NHS with research activity". For the first time, each Regional Health Authority (RHA) will have to draw up research plans and show the NHS management that these are being carried out.



Health research funding in England 1989/90
Source: UK Department of Health.

Most NHS-funded research projects now are aimed at assessing the effectiveness of different treatment methods. This will be stepped up, says Peckham, to weed out ineffective methods. The NHS will also keep a "weather eye" on fundamental biomedical research, looking for advances that may yield new treatments, he says. But sceptics point out that new and more effective treatments are often more expensive, which may dissuade hospitals from introducing them. Under NHS reforms introduced last month, health authorities can choose to send patients to the hospitals that seem to offer the best value for money. As the health authorities have limited budgets, there are fears that there will be pressure to send patients to hospitals where treatment is cheapest.

A new central NHS research and development committee is to be appointed in the coming months, and will set between six and ten 'priority areas' for NHS research. RHAs will then bid for grants from a central pot of money to support these research areas. This centrally directed fund should ensure that the separate research plans put together by the RHAs fit within the national strategy, says Peckham.

The first job, however, is to find out just how much the NHS now spends on research — a figure now known only approximately. Peckham estimates it to be about £225 million a year (about 1 per cent of the total NHS budget), but freely admits that NHS research spending has never been calculated accurately. The government plans to increase spending on research to 1.5 per cent of the NHS budget within five years.

Peckham also wants to increase collaboration between the NHS and other British bodies funding medical research. He aims to set up several research liaison groups, to bring together representatives from the NHS, the Medical Research Council, the medical research charities, industry and the universities. Each liaison group will concentrate on a specific health problem, such as cardiovascular disease or mental health. Peckham's model is the UK Coordinating Committee on Cancer Research, run by the Medical Research Council and several of the leading cancer research charities, and widely praised for its role in coordinating British cancer research.

Peckham's appointment to the new post of NHS director for research and development followed criticism from the House of Lords Science and Technology Committee of the low priority given to research in the NHS. **Peter Aldhous**

IVORY TRADING

African states invoke origin test to resist ban

Johannesburg

SOUTH African states are planning to market culled ivory which has had its origin certified using a technique described last year in *Nature*. In principle, the technique can ensure that ivory does not come from poached elephants and thus protect Africa's elephant population, while still making some ivory available for sale.

Five states (Botswana, Namibia, Zimbabwe, Malawi and Zambia) are concluding plans to establish a Southern African Ivory Marketing Centre (SACIM), which will auction ivory at a market in Gaborone. They hope to persuade the Convention on International Trade in Endangered Species (CITES) to exempt certified ivory from the world ban on trade in this commodity.

When CITES decided in October 1989 to declare a two-year moratorium on the ivory trade, it angered Botswana, South Africa and Zimbabwe, which derive considerable income by exporting ivory culled from their populations of African elephants. The populations in these countries, in which poaching is largely under control, have been growing even while the elephant population across Africa as a whole has been in decline — from about 1.34 million individuals in 1979 to 622,000 today. Botswana, Zimbabwe and Malawi entered immediate reservations to the ban, but these are of little avail so long as importers (particularly Japan, which imported almost half the world's ivory in 1989), observe it.

Ivory is currently being stockpiled in South Africa, as culling programmes in the Kruger National Park (which contains most of its elephant population), are continuing. The National Parks Board will lose an estimated R6 million in revenue over the two-year period. Zimbabwe and Botswana have probably suffered considerably higher losses, and are far more strapped for cash.

Until recently, there had been no way of determining whether ivory originated from a country where culling is permitted or from one where elephants are in danger of extinction. But the authors of two recent papers in *Nature* (346, 744–749; 1990) provided a solution: by measuring the isotope ratios of carbon, nitrogen and strontium in samples of ivory and bone, different isotopic "signatures" can be determined for elephants from different localities.

Botswana's deputy director of Wildlife, Nigel Hunter, says that part of SACIM's revenue will be channelled back into elephant conservation programmes.

Michael Cherry

REMOTE SENSING

ESA's eye on the oceans

London

THIS month, the European Space Agency (ESA) should launch ERS-1, the first stage of ESA's Earth observation programme, and the most ambitious package of remote-sensing microwave instruments yet put into orbit. The satellite will provide a wealth of oceanographic data, which will help to improve climate prediction models, but controversy still surrounds ESA's policy on setting prices for those data. Except for some 200 scientists chosen by ESA as "principal investigators" for the mission, academic researchers will be charged for ERS-1 data at the same prices as those fixed for commercial users.

ERS-1, which will fly in a polar orbit, was due for launch on 3 May, from Kourou, French Guyana. But on Monday this week, problems with the third stage of the Ariane-4 launch rocket had set the launch date back, possibly by more than a week.

The key component of the ERS-1 payload is the Active Microwave Instrument (AMI). Because clouds are transparent to microwaves, this will give an uninterrupted view of the Earth below. In its Synthetic Aperture Radar (SAR) mode, the AMI will produce detailed radar images of a 100-km-wide strip of the Earth's surface. These images will be used in land-use surveys, to monitor oil slicks at sea, and may allow oceanographers to detect ocean fronts, where bodies of cold and warmer water meet.

SAR equipment is not new in space. In 1978, the US National Aeronautics and Space Administration (NASA) Seasat satellite included such a radar device. But Seasat gave only a tantalizing hint of the full possibility of SAR data: the satellite suffered a massive power failure after only 100 days in orbit. The launch of ERS-1 is part of a new surge of interest in SAR imaging. The Soviet Union launched a satellite fitted with SAR earlier this year, and the SAR-equipped Japanese Earth Resources Satellite is due for launch in early 1992.

Unlike the Soviet satellite, ERS-1 will do more than produce SAR images. For most of the time, the AMI will operate in its 'wave' mode, taking snapshot images of a 5-km square every 200 to 300 km along the ocean surface, or in the 'wind' mode, measuring the backscatter from radar beams angled at 45 degrees to the sea surface. This backscatter can be used to calculate wind speed and direction at the sea surface. These data will be a key input to the World Ocean Circulation Experiment, the largest international collaboration in oceanography.

ERS-1 will also carry a radar altimeter, to measure variation in sea level and the height of ice sheets, and the Along Track Scanning Radiometer, which will measure the water content of the atmosphere and sea surface temperature.

ERS-1 has such an impressive range of hardware in part because ESA spending is decided by industry ministers from ESA member states, which are willing to pay into ESA's space programmes because their space technology industries benefit from the manufacturing contracts that result. But this commercial mindset also influences ESA's data policy, so that academics will be charged the same for ERS-1 data (several hundred pounds for each SAR image) as any other user.

This rigid commercial policy has been pushed most strongly by the British. But some relaxation, perhaps by allowing academics working on environmental research cheaper access to data, is expected for ESA's future remote-sensing satellites. NASA, which has a stronger interest in research than ESA, already takes this line (see *Nature* **346**, 600; 1990). The difficulty of selling environmental remote-sensing data is likely to be a major factor in ESA's future data policy.

Many ESA officials say it is not possible to recover costs by selling remote-sensing data — a view which is supported by the disappointingly low number of applications for data received by the commercial US Landsat satellite.

Peter Aldhous

TECHNOLOGY POLICY

Materials top critical technologies list

Washington

ANSWERING **a congressional plea for the US government to take the lead in setting technology priorities, the White House last week released a list of 22 'critical' technologies.**

Separated into six general categories — materials, manufacturing, information and communications, biotechnology and life sciences, aeronautics and surface transportation, and energy and environment — the list is heavy on those technologies related to economic prosperity and national security and light on basic research and exploration technologies.

Topping the list are five materials technologies, reaffirming the high priority the Administration places on that field. Earlier this year, White House Office of Science and Technology Policy (OSTP) officials previewed a large, multi-agency materials research initiative that the science office is planning for the 1993 budget (see *Nature* 350, 365; 4 April 1991).

Computers dominate the rest of the list, with seven key technologies in the category of information and communications. The White House panel's selections were: software; microelectronics and optoelectronics; high-performance computing and networking (also the subject of a new OSTP funding initiative); high-definition imaging and displays; sensors and signal processing; data storage and peripherals; and computer simulation and modelling.

Biomedical research was represented in only two technologies: applied molecular biology and medical technology.

OSTP is establishing a critical technologies institute to ensure that government research spending reflects the priorities of the list. Following a Bush Administration mandate, government support will be limited to 'generic, precompetitive' technologies, to avoid being put in the position of picking industry winners and losers.

Christopher Anderson

ASTRONOMY

Keck telescope twin gets the go-ahead

Washington

KECK I, which at 10 metres was to be the world's largest telescope when completed next year, has a competitor for the title. The W. M. Keck Foundation announced last week that it has committed $74.6 million to build an identical 10-metre telescope, Keck II.

Like Keck I, the new telescope will be built on the 13,600-foot Hawaiian volcano Mauna Kea by a consortium of Caltech and the University of California. To allow optical and infrared interferometry, the new telescope will be built adjacent to Keck I. Separated by a distance of 85 metres, the two telescopes will have the effective resolution of a telescope with an 85-metre mirror — an unparalleled combination of light-collecting power and spatial resolution for a ground based telescope. Keck I will be completed next year; Keck II is scheduled for completion in 1996. They will be joined on Mauna Kea later in the decade by a Japanese 8-metre infrared telescope that was approved earlier this year.

Christopher Anderson

Correction: Gamma-Ray Observatory

LAST month's account of the launching of the Gamma-Ray Observatory (GRO) (*Nature* **350**, 451; 1991) contained a concise and accurate description of a grazing incidence photon detector. Unfortunately, such devices work only for X-rays, and GRO has no such thing on board. The two large instruments, EGRET and COMPTEL, are based on scintillators. In EGRET, energetic gamma-rays create electron-positron pairs, whose energy and tracks are used to reconstruct the direction of origin of the incident gamma-ray. In COMPTEL, photons from successive Compton scatterings of a gamma-ray achieve the same purpose.

Gulf war and sanctions

SIR — On the day Iraq invaded Kuwait, I completed my thirteenth year as an associate professor at the medical school of Kuwait University. I was one of a group of expatriates who joined the university in 1977 and with the Kuwaiti staff helped to establish the medical school.

During the past 13 years, we watched the medical school grow into a well-established scientific institution. It has always set very high standards for both students and research. This was recognized by many international institutions as well as by scientists and academic visitors.

The medical school also established a sound and well-designed postgraduate programme. This was possible because of excellent planning and the availability of up-to-date equipment and materials as well as a good infrastructure.

The medical school had held an international scientific meeting every three years and symposia and workshops were also held every year in all branches of basic and clinical sciences and the proceedings of these meetings were published. This scientific activity re-emphasizes not only the faculty's reputation for outstanding original research but also that the research is directly applicable to the needs of Kuwait and the Gulf region.

The scientific community all over the world was shocked and appalled by the looting, destruction and removal of virtually every piece of equipment and materials from scientific and educational institutions in Kuwait.

Nothing similar had ever been seen anywhere in the civilized world. It is well known that Saddam Hussein's regime is despotic and tyrannical and rules by fear. But what I cannot comprehend is the dishonesty and lack of scientific decency of the Iraqi professors who were personally involved in the systematic dismantling of the various scientific institutions around Kuwait (see *Nature* **347**, 420; 1990 & **349**, 450; 1991).

I strongly believe that the scientific community should impose sanctions on scientific contributions from Iraq for some time to come, to force Iraq to pay for the equipment that was removed from Kuwait.

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SIR — While it seems unlikely, from the evidence reported, that the Iraqi 'baby milk' factory was a biological weapons assembly plant (see *Nature* **350**, 117; 1991), use of the factory for the production of the bacteriological media required for the production of such weapons is a very real possibility. Peptone powders and more complex formulations could be easily produced and shipped to a separate facility for cultivation of the microorganisms used in the manufacture of biological weapons. The apparent lack of microorganisms or their products at the Iraqi site does not exclude the possibility that the factory was indirectly involved in the production of biological weapons.

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Infectious paradigm

SIR — Walter Gilbert's vision (*Nature* **349**, 99; 1991) that the paradigm shift provoked by the human genome project may free small science from DNA technology is convincing, but is there not a danger that the same paradigm will be applied to small science itself? So much can be seen in the criteria used in the determination of research grant applications — the certainty of a promised result, and of an advertised timetable, for example.

What is happening is that biologists are muddling together two activities which, in the physical sciences, are separate — scientific and engineering research. Both demand ingenuity and perseverance, but they are different.

Research in physics, striving for generalizations, reflects the curiosity of individuals, cannot be totally planned and does not guarantee interesting results. Engineering research, by contrast, seeks information specific to one group of phenomena that can then be put to practical use. The work is varied — the thermal conductivity of ceramics or the execution time of algorithms — and usually involves social or even corporate interests, but the methods are known, the timetables calculable and the outcomes foreseeable.

Traditionally, that difference has been reflected in the practice of funding agencies. Engineering projects have been awarded contracts, science research projects have won grants.

Medicine, the application of biological knowledge to the preservation of human health, is biology's analogue of engineering. None of us disputes that the genome project will benefit medicine, but we should not lightly assume that all of the coming decade's addition to our understanding of biology will be a by-product of molecular medicine. And we should beware of unwittingly subjecting biologists to selection pressures that only engineering research can meet.

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British science

SIR — Terence Kealey (*Nature* **350**, 370; 1991) raises two interesting issues about the structure and performance of British science, both of which relate to studies undertaken by the Science and Engineering Policy Studies Unit (SEPSU) in recent years.

One issue concerns the interpretation of bibliometric data, on which some of Kealey's comments are misleading. First, the Institute for Scientific Information (ISI) did indeed increase its journal coverage by nearly 40 per cent between 1975 and 1982, but one cannot infer from this that the global population of journals also increased by 40 per cent, still less that Britain "increased its numbers of papers by some 30 per cent". The 40 per cent figure is primarily a statement about ISI, not about world output. Second, we at SEPSU showed in our original study that both the 1973 fixed journal set and a larger set defined in 1981 give the same figure — 8.3 per cent for the UK share of world output in 1982. We did not 'ignore' the journals established after 1973: we demonstrated that they did not materially alter our conclusions. Third, to the extent that research is a competitive business, market share matters as well as absolute volume.

The second and more important issue is the extensive change to the structure of British science that has occurred over the past 10–15 years. Kealey very rightly draws attention to the 'privatization' of British science. One consequence of this has been a switch from long-term to project funding and, with it, the rapid growth in the number of short-term academic staff that Kealey mentions. However, it is unsafe to draw too many comforting conclusions from this development: nearly two-thirds of these short-term academic staff are below PhD status and would not normally be regarded as independent researchers, while the remainder have uncertain career prospects. Moreover, while it is certainly true that industrial and charitable funding of university scientific research has doubled in recent years, it still constitutes only 35 per cent of total external funding of university research, or 18 per cent of UFC + external funding.

Drawing in part on earlier SEPSU work, the Royal Society is now conducting a major inquiry into long-term issues in science policy, to think through the implications of the above, and other, structural changes and to identify some of the policies that will be needed to sustain the health of British science into the next century (see *Nature* **349**, 183; 1991). Anyone wishing to contribute to the inquiry is invited to write to the president, Sir Michael Atiyah (The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG).

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Wittgenstein and reality

SIR — It is quite understandable that a gung-ho positivist, who unquestioningly accepts what he sees as reality, is annoyed by someone such as Wittgenstein and wants to rubbish him. However, the grounds selected by J. R. Smythies (*Nature* **350**, 9; 1991) who says that Wittgenstein was talking 'schizophrenese' — a form of speech disorder characterized by the fact that the meaning of a schizophrenesiac proposition is not wholly contained within it — will not do.

Of course, it would be easy to reject anything we do not understand as a distorted proposition or a non-proposition, and this is just what Wittgenstein, in common with the logical positivists, tried to do in the *Tractatus*. However, as Wittgenstein's later work (especially the *Philosophical Investigations*) shows, no proposition or set of propositions wholly contains their meaning. An understanding of the meaning of a proposition always depends upon other, external, factors. We understand one another — for example, I understand Smythies and Smythies understands his patients — because we share a common set of assumptions with which to interpret our communications. Lacking such a mutual basis we must seek it out and fail to find it before we can dismiss propositions under such a rubric as 'schizophrenese'.

It might indeed be thought that to reject an investigation of the meaning of scientific statements (which is the business of philosophy), while confidently accepting their validity, is more truly a sign of a schizophrenic (shattered) view of reality.

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SIR — J. R. Smythies' explanation and dismissal of Wittgenstein's philosophy is that he was a schizophrenic writing poetry in German. That necessarily implies an admirably humble confession of a difficulty in grasping some of the illuminating aphorisms, yet it is as helpful in evaluating Wittgenstein's thought as the comment on a recent esoteric French philosopher, that he died of AIDS.

Turning the tables on Smythies, one can note that his letter reveals his quasi-religious paradigmatic belief that the neurosciences are those "from which ... our only true understanding of the mind can come". Wittgenstein saw philosophy as therapy for such misplaced confidences. Perhaps he would have thought of Goethe, Shakespeare, Cervantes, Kafka *et al.* This is no denial of the validity and achievements of scientific enquiry. There is, however, a warning that much thought, as is most especially true for example of mathematical creations, may depend on axioms utterly obvious but only to whole generations influenced by Zeitgeister. Extrapolating from what is successful for some issues to everything is at least questionable. Is it too germanic to note that mysteries about Being remain, or to wonder whether there is something wrong in the basic formulations we use in trying to grasp how conscious experience could evolve from matter as we have to conceive it?

Wittgenstein was undoubtedly a very disturbed and distraught person but in the light of psychiatry's own difficulties with the word schizophrenia, is much achieved by a diagnosis in this context? I must, of course, confess my own insecure and heretical position. It stresses the personal politics involved in indicating the similarities or differences between us and those called schizophrenic. That is done in part to emphasize either our ability to empathize or their strangeness. Much that many of them say is to some of us instructive as they are to a significant degree defined by their apparent emotional need to stand painfully outside complicity with the common sense of the period. Perhaps it is appropriate here to quote Karl Jaspers (1946), who went a long way to define who are schizophrenics. They are those whom among other things, he said, we cannot understand (*Verstehen*) humanly so must explain (*Erklaren*) scientifically. Nevertheless, he added (much nearer to my views), "extreme psychotic states offer a parable — patients see into depths which do not belong so much to their illness as to themselves as individuals with their own historical truth ... in psychotic reality we find an abundance of content representing fundamental problems of philosophy .... The philosopher in us cannot but be fascinated by this extraordinary reality and feel its challenge."

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Jaspers, K. *Allgemeine Psychopathologie* (Springer, Heidelberg, 1946).

Cancer—EMF connection

SIR — *Nature* has recently (**349**, 554; 1991) discussed the cancer–electromagnetic field connection. When two phenomena correlate, but no obvious direct linkage exists, it is worthwhile asking whether an indirect linkage may exist.

Cancer is well know to correlate with action of highly reactive chemicals on tissue. The presence of certain types of electrical systems can result in the production of highly reactive chemicals. Thus, for example, high-voltage transmission lines can be expected to produce ionization of air as a result of discharges, and production of low levels of materials such as ozone and nitrogen oxides. Similarly, if electric hair-driers have motors with brushes, the sparks at the brushes could have similar results, while the high voltages used in television sets for the cathode-ray tubes could potentially do the same.

The reactive gases would have a relatively high local concentration near the device that produces them and so potentially account for the reported correlations. Could there be a correlation between the use of heavier wiring and large motors (because of starting currents)? This could again help produce the unexpected correlation.

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Health risks

SIR — David Lindley (*Nature* **346**, 507; 1991) makes the valid suggestion that public fears about health risks could be mitigated if scientists presented statistical arguments more precisely so that the public understood them better. In passing, however, he appears to give credence to the popular misconception that nuclear power is 'safe' compared with not wearing a motorcycle helmet or seat belt. But in terms of unit risk of death, the converse is true. Motorcycle helmets and seat belts have not been proved to reduce individual risk of death (and probably do not, see J. G. U. Adams *Ergonomics* **31**, 407–428; 1988), whereas nuclear power is known to have killed.

This example illustrates the important point that statistical arguments are futile when addressing public health fears because the objective statistical information is simply not available in matters of national policy. Where, for example is the evidence that crash helmets and seat belts 'save lives'? These unproven devices are promoted as beneficial (in the absence of evidence) and thus deflect public attention from the fact that a transport policy that encourages private road transport (responsible for 5,000 accident fatalities a year in the United Kingdom) is inherently dangerous. In similar vein, energy efficiency, although cheaper and inherently safer than nuclear power, is not national policy and thus not promoted. People certainly often appear to be 'penny-wise and pound-foolish' in their personal risk perception. But underlying this actuarial schizophrenia is the uncomfortable reality that public policy often dictates the risks the public are forced to take. The public, of course, orders its fears accordingly.

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Funding of molecular biology

Benno Müller-Hill

The way that molecular biology is funded in Germany is damaging the universities. German policy makers should follow the advice of Wilhelm von Humboldt, and concentrate teaching and research in the universities.

MONEY for fundamental research in molecular biology is getting tighter. Only one in ten of the grant proposals put forward today by newcomers is funded by the National Institutes of Health in the United States. Although the situation is better in Europe, the strain is noticed there too. And it is felt differently in different countries and institutions. Germany is interesting in this respect because its fundamental research is divided into two parts.

On one side are several research centres such as the Max-Planck-Institutes or the German Cancer Centre. The directors of these institutions receive ample ground support and can, in addition, apply to the government for grants. But they do not have to prove their productivity repeatedly to receive this support — it will be given to them from their appointment until their retirement. A convincing argument in favour of this procedure is that it encourages truly outstanding researchers to attack fundamental problems, which often take several years to solve. also, only research centres can afford extremely expensive equipment.

On the other side are the universities. Most German university professors get little or no ground support for their research. That which they do receive just suffices to fund practical classes and small libraries. Thus, almost all of the money for research in most universities comes from granting agencies. The largest such agency in Germany is the Deutsche Forschungsgemeinschaft (DFG). Its budget of more than DM 1,000 million is as large as the budget of the Max-Planck-Gesellschaft (MPG), the society which funds the Max-Planck-Institutes.

This has not always been so. The number of scientists has grown exponentially since 1700, doubling about every 15 years. Thus only 1.5 per cent of the present number of scientists existed in 1900. At that time, all research in biology and chemistry in Germany was done exclusively in university institutes or in industry. State-financed research centres did not yet exist in these two fields. This was due to the effort of the founder of Berlin University, Wilhelm von Humboldt, who had fought successfully against research in academies and for the unity of teaching and research within the universities.

This changed in 1911 when German Kaiser Wilhelm, well known for his sense of humour, used the centennial of Berlin University to announce the foundation of research centres outside the universities with the financial support of industry. These Kaiser-Wilhelm-Institutes were small to begin with. The best of the university professors were hired as directors, who rarely had more than three postdoctoral researchers and very few graduate students. This enviable situation did not last long; the First World War began and ended in disaster for Germany.

The abysmal economic situation after the First World War was the proper occasion for the foundation of the Notgemeinschaft Deutscher Forschung in 1920, the predecessor of the present DFG. Its specific goal was to help university research. It introduced peer review and two-year grants. At the end of the Weimar Republic, about 90 per cent of its money for biology went to university institutes. As industrial money vanished, the Kaiser-Wilhelm-Institutes needed and got more and more money from the state.

Mary Evans

Wilhelm von Humboldt (1767–1835)

When the Nazis came to power, they removed all Jewish scientists, but they did not abandon the remaining German ones. In the absence of notable inflation they more than doubled the budget of the Kaiser-Wilhelm-Gesellschaft from 1932 to 1940/44 and they increased the DFG grant for fundamental biology fivefold from 1932 to 1940/44. They centralized the review system of the DFG and changed the distribution of the money: between 1940 and 1944, about half of the DFG grant for biology went to Kaiser-Wilhelm-Institutes. The Kaiser-Wilhelm-Institutes clearly prospered financially during the regime of the Nazis.

After the Second World War, Germany was in shambles. The Kaiser-Wilhelm-Gesellschaft was recreated as the MPG in 1948. From this time on it relied almost exclusively on state money. The former Notgemeinschaft was recreated too as the DFG, actually under the name the Nazis had given the institution but with the old rules from the Weimar Republic. Today both organizations accept and spend about the same amount of money received from the state, and more than half of all German research in molecular biology is done in research centres, assuming that the number of Germans in the European Molecular Biology Organisation is representative of this.

Nobody knows how long exponential growth in the number of scientists will go on. At the moment about one per cent of the population of the United States could be called scientists (that is, they have a low science degree). But there has not been a proportional growth in the number of top-

rate scientists. It is an illusion that all directorships of the Max-Planck-Institutes can now be filled by people who will be as productive as Butenandt, Kuhn, Meyerhof or Warburg, to mention just a few Nobel prize winners from the first half of this century. The MPG can now attract all the best and most of the better molecular biologists.

The universities can no longer compete in Germany. There have been very few cases of university professors who have turned down offers of appointments made to them by the MPG. Most university professors are more than happy when they can exchange their poor university chair and uncertain grants for a Max-Planck position, and reverse traffic of directors from the Max-Planck-Institutes to the universities is almost unheard of. It is true that universities can get good researchers from the Max-Planck-Institutes, but the research centres offer strong incentives for outstanding researchers to stay with them as permanent members or as division heads. This situation is beginning to damage the German universities. If more and more of the better research is coming from the Max-Planck-Institutes and other such centres, then their representatives will begin to argue that their funding should increase. This will inevitably lead to the collapse or reshaping of the German universities: they will become places where undergraduates are taught by professors who do not do research, and graduates will move to the centres to do the research for their doctorates.

Is this a bad prospect? History has already informed us of the answer. The United States uses a grant system based on the German Notgemeinschaft or DFG to fund its flourishing research, which is conducted mainly in state or private universities. The Soviet Union has opted for the Kaiser-Wilhelm system by concentrating almost all of its research in Academy Institutes. If the Academy Institutes in Moscow are compared with the universities in Boston it is beyond question who is the winner in molecular biology: the Americans. The few seminal discoveries made at Academy Institutes never came from the ground. The directors of the Academy Institutes, with between 200 and 500 graduates to oversee, were simply unable to recognize and promote outstanding individuals in time. Of course it can be argued that the one-party system with its effective secret police and its ineffective planning undermined all sincere efforts. This is certainly true, but I suspect that the main defect is elsewhere: in the near absence of functioning universities, the monopolistic research-centre system inherently performs very badly.

The Institute for Genetics, Cologne

The failure of the Soviet research centres is striking, but are not the Max-Planck-Institutes much more productive than comparative university institutes? To answer this question, my collaborator H. Herbertz and I have compared and evaluated Max-Planck and university institutes which are active in research in molecular biology or biochemistry. The table compares two Max-Planck-Institutes and my own university institute. My university institute — it was at the time the largest and richest in Germany — did at least as well if not better than each of the two Max-Planck-Institutes in terms of the cost of articles and citations or the number of citations per article. The data presented indicate that research at a comparable university institute was effective when it had full support through the competitive grant system of the DFG.

The universities are productive in that they train the future experts for industry and the research centres. Industry and the state will suffer if further growth of research centres catalyses the destruction of the universities. To prevent this, the research centres could support outstanding university institutes by grants. German policy makers should follow the advice of Humboldt and concentrate teaching and research in the universities. Moreover, they should increase strongly and steadily funding of the universities and their granting agency, the DFG, and slow down or even freeze further growth of the research centres. □

COMPARISON OF THREE GERMAN RESEARCH INSTITUTES

	MPIB	MPIMB	IGUC
Permanent budget in 1982 (DM million)	32.0	12.5	4.9
Grants in 1982 (DM million)	2.9	1.3	4.4
Total budget in 1982 (DM million)	34.9	13.8	9.3
Laboratory space (m^2)	20,434	5,400	2,883
Doctoral dissertations	72	43	33
Articles	629.2	370.0	168.0
Cost of articles (DM thousand)	55.5	37.3	55.6
Citations	7,016.2	3,906.9	3,839.0
Citations per article	11.1	10.6	22.8
Costs of citations (DM thousand)	5.0	3.5	2.4

Indicators of the scientific productivity of the Max-Planck-Institute for Biochemistry in Munich-Martinsried (MPIB), the Max-Planck-Institute for Molecular Genetics in Berlin (MPIMG) and the Institute for Genetics of the University of Cologne (IGUC), 1980-1984. Yearly budgets are as follows. MPIB and MPIMG for 1982, data provided by the Generalverwaltung of the MPG. The sum does not include the costs of administration. Grants provided by DFG through Normalverfahren are not included either. IGUC for 1982, data provided by the administration of Cologne University. The sum includes the salaries of all employed, teaching costs, research costs, electricity, heat, maintenance, and so on. It does not include central administration or other central costs. All DFG grants and grants of the Bundesministerium für Forschung und Technologie (BMFT) are noted. Number of doctoral dissertations finished between January 1980 and December 1984 according to *Jahrbuch der MPG* and information of the Dean's office of Cologne University. Articles: the yearbooks and reprint collections of the various units of the various institutes were comprehensively consulted. All articles including short notes, abstracts, articles in books, education reviews which were cited at least once by other researchers were counted. These articles were weighted in the following manner. When two groups collaborated they were counted as 0.5, when three groups collaborated as 0.33, and so on. Citations: the *Citation Index* of 1980-1984 and 1985, 1986, 1987 and 1988 were consulted. Misspellings and umlauts were considered. Citations of papers were collected for the year of appearance and for four further years. These citations were weighted in the following way. When two groups collaborated, and the Munich, Berlin or Cologne group provided the first author, the number of citations was multiplied by 0.66, when these laboratories did not provide the first author, the citations were multiplied by 0.34. When three groups collaborated, the citations were multiplied by 0.5 and 0.25 respectively.

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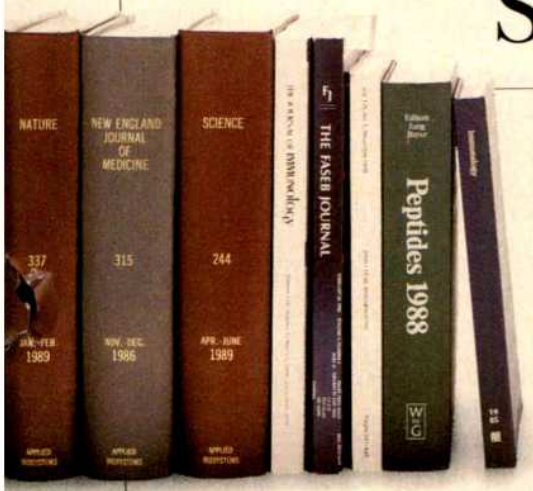

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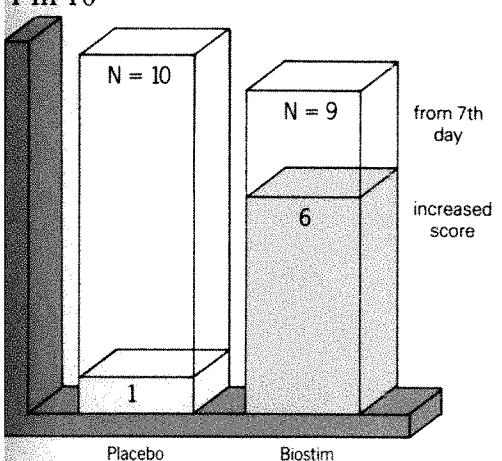

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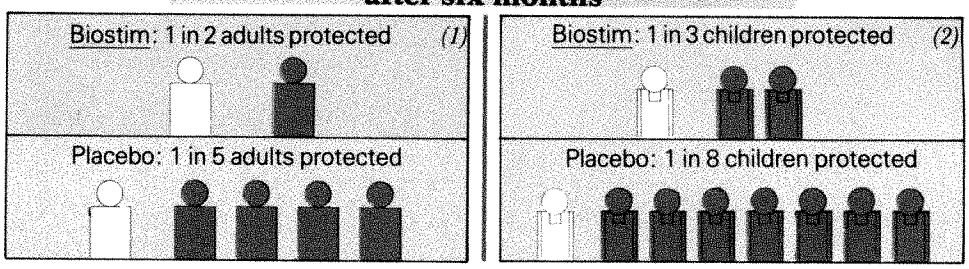

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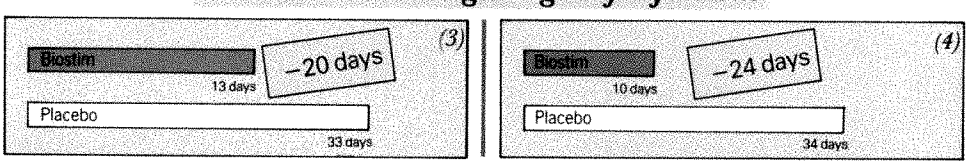

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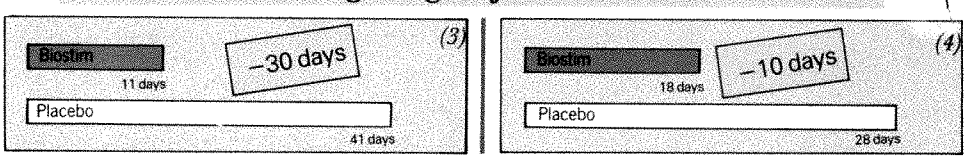
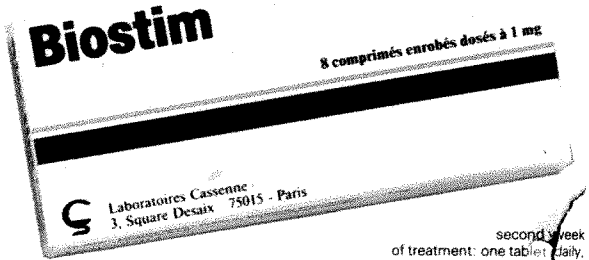


(1) Prof. Chrétien 52 cases — Ref. Rev. Pneumol. Clin., 1985, 41, 3: 213–217
(2) Prof. Pech 78 cases — Ref. Cahiers ORL, 1987, XXII, 3: 217–220
(3) Prof. Boutin 73 cases — Study over 9 months, Ref. Poumon Coeur, 1983, 39: 53–57.
(4) Prof. Paupe 42 cases — Study over 6 months Ref. Ann. Pédiatrie, 1986, 33 (9): 843–845



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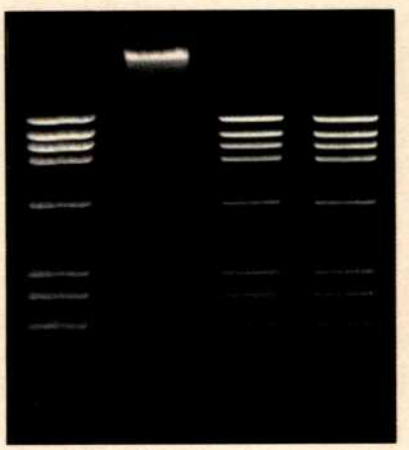

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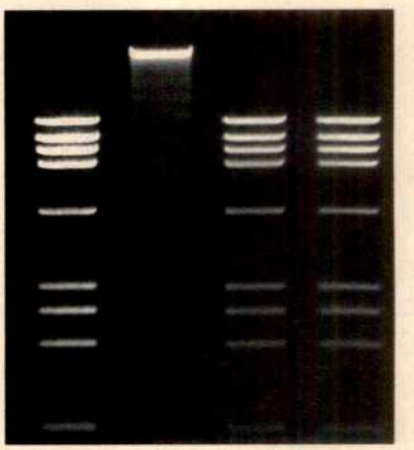

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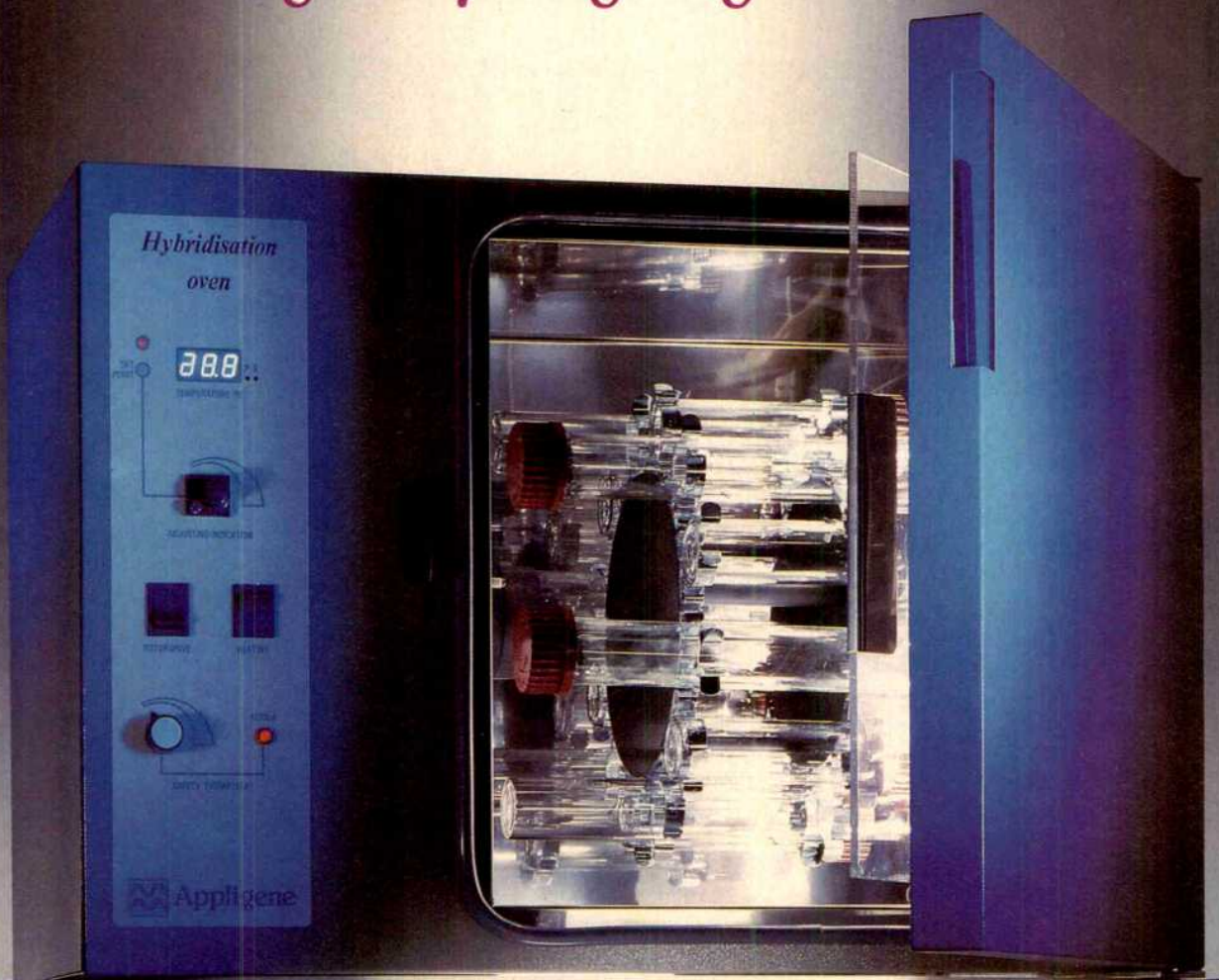

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Another mountain from a molehill

Stealing ideas is bad for research and the reputation of science, but so are unfounded allegations that there has been a theft when there is none, now put in circulation by a materials scientist from Pennsylvania.

PLAGIARISM is the most serious of the known crimes against scholarship. Not only does it confuse the record by making it impossible to tell who exactly said what, but it amounts to the literal theft of another's words, thereby depriving the victim not merely of the credit for the content of the stolen words but of whatever thought and imagination they embody. It is a shabby business.

So what about the appropriation of others' ideas? That is also shabby, whence the convention that authors should be meticulous in their references to the antecedents of their research. By failing to refer to a significant earlier article, an author misleads his readers about the genesis of what he or she has to say and also captures an undue proportion of the credit for what is new. Oversights of this kind, deliberate or otherwise, are the most common cause of disputes about priority. People will even hotly claim precedence for ideas recognized to be hare-brained, but even that is understandable.

In short, the omission of crucial references is reprehensible, may amount to the theft of other people's ideas and, as far as possible, should be stopped. The excuse that 'there is no copyright in ideas' will not wash. But there are obvious difficulties. What about, for example, when two groups of people make more or less the same discovery at more or less the same time and submit accounts for publication separately? One may accuse the other of having picked up crucial information at a meeting, or may even suspect that referees have spilled the beans.

Obviously, some oversights can also be innocently explained; an author may not have known of earlier related work. A recent article on this page (*Nature* **349**, 363; 1991) which drew attention to a recent reassessment of the importance of the expansion of the Universe and its age in the resolution of Olbers' paradox has, for example, already drawn the comment that a similar conclusion was reached in a contribution to *Nature* nearly 30 years ago.

Such incidents, while still regrettable, are likely to become more common. However the bibliographic databanks are embellished, there is a high risk that anticipatory work will be overlooked, at least in fields not constantly reworked by the textbook and monograph writers. As the record on which contemporary research is built stretches further back, such occurrences will multiply, at least until the bibliographers learn to index their material by concept as well as content.

Most people accept these difficulties — but not, it appears, Professor D. M. ('Rustum') Roy of the Materials Research Laboratory at the Pennsylvania State University, who is well-known as a proselytizer of materials science. Roy is now deeply offended that *Nature* has published an article that fails to refer to two earlier publications (also in *Nature*) in 1971 and 1974 with which he had been associated. Ordinarily, the first step in such a saga is a letter of protest to the offending journal, which, fair play, Roy sent on 13 March, but intemperately. Most of what follows is concerned with what has happened since.

The article about which Roy protests is that by Patricia A. Bianconi, Jun Lin and Angela R. Strzelecki (*Nature* **315**, 349; 24 January 1991). Piquantly, it may be thought, the authors are also at Pennsylvania State University, but in the chemistry department. They point out the potential importance of synthetic materials in which inorganic constitutents are precipitated within an organic matrix and describe the precipitation of CdS crystals within a synthetic organic matrix consisting of polyethylene-oxide.

Nobody would mistake the article for a claim to have made a usable material; its chief interest, as explained by Stephen Mann in an accompanying article (*Nature* **349**, 285; 1991) is that the authors have been able to find conditions under which apparently amorphous CdS makes a phase transition to a cubic form, suggestive of how the mineral components in materials such as bone and teeth are often orientated and otherwise disposed so as to enhance their links with the underlying matrix. Appropriately, but, as events have shown, perhaps unfortunately, the authors use the terms 'biomimetic' and 'biocomposite'.

Roy has taken up the cudgels on behalf of two earlier articles. One describes the formation of porous bone-like structures by using a resin to make a cast of the pores in natural bony material (the skeleton of the echinoderm known as the slate-pencil urchin) in which ceramic materials are then precipitated (J. N. Weber *et al. Nature* **233**, 337; 1971). The other describes a technique for replacing the carbonate of coral by phosphate to form harder apatite (D. M. Roy & S. K. Linnehan *Nature* **247**, 220; 1974). Both principles have since been used in the fabrication of synthetic composites.

Roy's cudgels have been exercised energetically. He has, for example, distributed widely an undated memorandum "in the interests of maintaining the integrity of the modern science enterprise" which says that, while this is not a case of "cheating, fraud, malice, etc.", *Nature* and the Pennsylvania State University have acted jointly in the overselling of science, which is being advertized "like cigarettes using inaccurate misleading exaggerations". The same document complains that *Nature* made things worse by inviting Stephen Mann to comment on Bianconi *et al.*

This would ordinarily have been but a storm in a teacup had not the document fallen into the hands of a news magazine to which Roy confided that he knew from a telephone conversation that the editor of *Nature*, correctly identified by first name, was annoyed ("pissed off" was the verb he chose) with Mann for having written an egregious puff, but that Roy did not know whether the outcome would be a scandal or a "cover-up". Yet in the only conversation on this or (if memory serves) any other subject, Roy said merely "Why would I say a thing like that?" It is mystifying, to say the least.

So is the feasibility of the remedies Roy advocates. He would, for example, require all publications to include an explicit statement of how the authors had searched the literature, that journals should be compelled to publish amendments to lists of references and that grant-making agencies should appoint ombudsmen to assess the validity of claims to novelty. (One little irony is that the two earlier articles do not use the word 'biomimetic', on which a literature search would have been conducted, but which may not have been invented in the 1970s.) There was even at one stage talk of having this apparatus given teeth (in the United States) by an act of Congress.

All of us will appreciate, all too often from first-hand experience, the acuteness of the sense of neglect evoked by recognizing our own thoughts converted by others into words, and the ferocity of the emotions that can be aroused when others occupy intellectual territory we regard as ours. (This, no doubt, explains Roy's assertion that using the word 'biomaterials' in connection with the precipitation of CdS in a polymer gel is tantamount to 'poisoning the language of science'.) Yet even by those tests, Roy's reaction to Bianconi is no less exaggerated than the supposed overselling of which he complains.

Consciously appropriating the ideas of others without acknowledgement is bad for science, but so is making a needless fuss about empty issues. Indeed, gratuitous unseemliness may be just as damaging, suggesting to others that researchers are incorrigibly petty and quarrelsome. **John Maddox**

CLIMATE FEEDBACKS

Limit to greenhouse warming?

Andrew J. Heymsfield and Larry M. Miloshevich

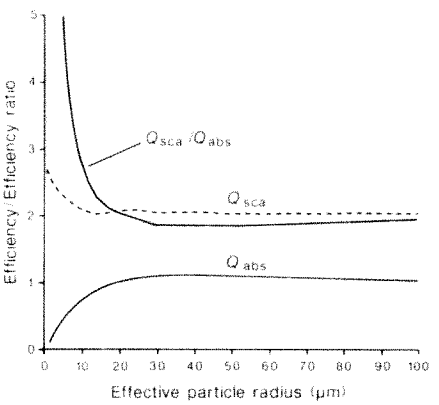
THE greenhouse effect, a net atmospheric warming attributable primarily to the absorption of infrared (longwave) radiation by water vapour and clouds, may be limited in magnitude in the tropics by a feedback mechanism involving sea surface temperature (SST) and cirrus ice clouds, as proposed by V. Ramanathan and W. Collins elsewhere in this issue (*Nature* **351**, 27–32; 1991). They find that when the SST exceeds about 300 K, vigorous, deep convective clouds rise to high altitudes and produce extensive cirrus ice clouds, reducing solar heating and limiting the SST to, at most, 305 K. In the absence of some such means to limit solar surface heating, the rising SST and consequent build up of water vapour in the atmosphere would result in a runaway greenhouse effect. This mechanism may have important implications for global climate change.

In the absence of an atmosphere, the fundamental factors controlling the planetary heat balance are the solar (shortwave) energy absorbed at the surface (the incident energy minus the energy reflected, or surface albedo) and the longwave energy emitted by the surface back to space. The atmosphere contributes to the heat balance through the absorption of shortwave radiation by atmospheric gases, addition of a cloud albedo to the surface albedo, and net longwave absorption by cloud drops and ice particles and by greenhouse gases (particularly water vapour, carbon dioxide and methane). The greenhouse effect is caused by the longwave absorption by atmospheric water vapour (and other greenhouse gases), and the net longwave absorption in clouds.

Ramanathan and Collins determine the upper limit of SST by correlating satellite radiation data with SST measurements over a period which includes the 1987 El Niño event, an occasional climate fluctuation affecting the southern Pacific area. They then establish values for the various radiative contributions to the Earth's heat budget.

The satellite data show a pronounced increase in the greenhouse effect as SST increases above 300 K. The authors find that the longwave absorption by clouds is the dominant contribution to the greenhouse effect, and interpret this observation as indicating an increase in cloud extent and optical thickness as SST increases. The data also show that cloud albedo increases as SST increases, thus the clouds that trap more longwave radiation are also more reflective to shortwave radiation. Furthermore, the data show that shortwave reflection increases with increasing SST at a faster rate than the corresponding increase in greenhouse warming. Eventually the increased cloud production provides sufficient shortwave blocking to inhibit further increases in SST, and prevents a runaway greenhouse effect.

Using the observed values for the rates of change of atmospheric and cloud contributions to the radiative heat budget, Ramanathan and Collins determine that the maximum possible tropical SST is between 303 and 305 K, in agreement with the maximum value of 304 K measured during the 1987 El Niño. The range of values given for the maximum SST is largely a result of the bounding values, taken to be 0.8 and 1.0,



The radiative effect of changing ice particle size: the 'Mie' scattering efficiency, calculated for spherical particles, is shown for shortwave reflections (at a wavelength of 0.65 μm; Q_{sca}) and for longwave absorption (at 10 μm wavelength; Q_{abs}). The ratio of these (Q_{sca}/Q_{abs}) is also shown (solid line). (Calculations by Patrick Minnis, Langley Research Center.) The effect of size on the radiative properties of ice particles could contribute to the feedback observed by Ramanathan and Collins, but *in situ* measurements of real nonspherical ice particles in clouds will be needed to clarify the details. This example, using one particular set of wavelengths, can only be taken as an indication of possible particle size effects on cloud radiative properties, as absorption efficiencies depend strongly on wavelength and multiple-scattering of shortwave radiation may also be important.

used to describe the fraction of greenhouse warming that is advected out of the tropics and is thus unavailable to affect the SST directly. These high values for the fraction of advected heat suggest that the feedback mechanism, which occurs in the warmest regions of the planet, may have consequences for the planet as a whole.

The mechanism that the authors propose to explain the data involves the effects of solar heating of the surface and resulting convection on the balance between greenhouse warming and cloud reflectivity. When the SST is below 300 K, the latent energy in parcels of moist air near the sea surface is insufficient to produce deep convection, but, when it exceeds 300 K, parcels have sufficient latent energy to rise to the upper troposphere as cumulonimbus clouds. At the cumulonimbus cloudtops, ice crystals form cirrus clouds spreading thousands of kilometres downwind, dominating radiative processes in the tropics when the feedback mechanism is operating. Because the optical depth of the cirrus clouds and their areal extent increase in response to increasing SST, the cloud albedo also increases, thus slowing and eventually preventing further increases in both SST and the associated greenhouse warming.

The authors suggest that increases in cirrus extent and optical thickness are responsible for the enhanced cloud albedo. It seems likely that changes in the distribution of ice particle sizes contribute to enhanced cloud reflectivity. The albedo of a cloud depends ultimately on the scattering behaviour of individual ice particles (the phase function, dependent on particle size and shape), integrated over both the particle size distribution and the path length through the cloud. Studies of stratiform clouds (A. J. H. & C.M.R. Platt *J. atmos. sci.* **41**, 846–855; 1984; see also the special 'FIRE' issue *Mon. Weather Rev.* **118**, 2259–2446; 1990) suggest that ice particles tend to be smaller at lower temperatures, with diameters of a millimetre or more near 0 °C, but only tens of microns at the temperatures characteristic of the tropical tropopause. This is a result of changes in diverse factors such as cloud water vapour content, cloud thickness, particle terminal velocity and vertical air velocity. These observations in stratiform clouds may not apply to the convective regions of the cumulonimbus; but, fallout of the larger particles with increasing distance downwind of the convective towers will leave cirrus clouds containing smaller ice crystals.

The effect of the size of ice particles on their radiative properties depends on their shortwave scattering efficiency (Q_{sca}) and their longwave absorption efficiency (Q_{abs}) for a given cloud volume and particle size distribution. The figure shows that, at least for spherical ice particles, the ratio $Q_{sca}/Q_{abs} \approx 2$ for particles larger than 25 μm, but the ratio increases significantly as their size decreases below 25 μm. As the SST increases, production of colder cirrus clouds may contain smaller ice particles which may lead to enhanced shortwave reflection relative to longwave absorption, in addition to the enhanced shortwave reflection caused by increases in cirrus ice mass alone.

It is unclear what relevance the feedback mechanisms proposed by Ramanathan and Collins have to the issue of CO_2 greenhouse warming: the climate's response to increases in atmospheric CO_2 could be very different from the negative-feedback response to solar heating that the authors describe. The source of the warming is the obvious difference. Whereas solar radiation directly heats the ocean surface, giving rise to the SST-limiting feedback, CO_2 produces warming in the overlying atmosphere through trapping of surface-emitted longwave radiation. As

most upper-tropospheric heating in the tropics is advected out of the region, CO_2 warming in the atmosphere should have much less of an effect on limiting the SST than does direct solar heating, although the relationship between atmospheric warming and SST is a subject of controversy. In addition, atmospheric warming by CO_2 may alter the vertical temperature profile, leading to changes in the atmospheric stability. □

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LIQUID CRYSTALS

Shedding light on alignment

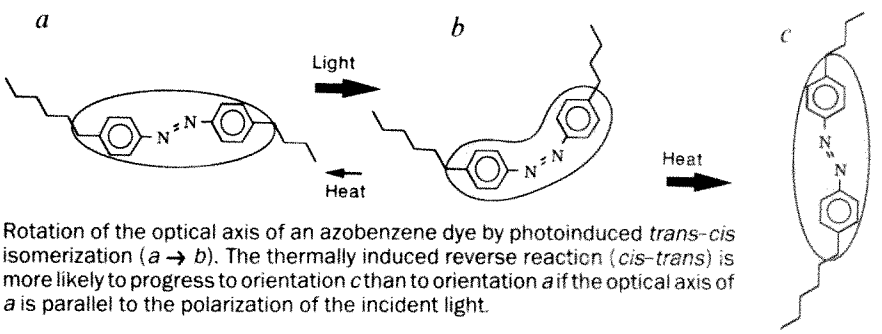
Cliff Jones and Sally Day

ORGANIC materials have enormous potential for use in electro-optic and opto-optic devices. Nowhere is this more evident than with liquid crystals, which are already familiar in the displays of digital watches and laptop computers. These devices are electro-optic: applying an electric field reorientates the liquid crystal molecules, thereby altering the device's optical properties. New effects and applications are being sought, and one particularly exciting possibility is that of controlling the molecular orientation using incident light. Such an effect is described by Gibbons *et al.* on page 49 of this issue[1], who show how to manipulate the orientation of a nematic liquid crystal by irradiating the surface alignment layer with polarized light.

Vital to most liquid crystal devices are surface alignment agents which define the molecular orientation of the device in the unswitched state. Numerous surfactants are available, but the usual commercial technique is to coat the inner surfaces of the glass cell containing the liquid crystals with a polymer such as polyimide. This causes the rod-like liquid crystal molecules to lie parallel to the surface. Rubbing the surface with a nylon cloth then imparts a preferred alignment for the liquid crystal molecules once the cell is filled. For example, in the common twisted-nematic device, the surfaces are rubbed at 90° to each other, so that the liquid crystal molecules twist through this angle from one surface to the other. Light is transmitted through the liquid crystals when viewed between crossed polarizers, and the cell appears light. Applying a small voltage (typically about a volt) reorientates the molecules, removing the twisted structure so that the device is dark. In this manner the twisted-nematic cell acts as a simple electro-optic shutter.

Gibbons *et al.* use a novel alignment layer that is a mixture of polyimide and an azo-dye. Anisotropic dyes of this type are often used in liquid crystal applications because they align parallel to the liquid crystal molecules, providing anisotropic absorption of incident light and allowing displays incorporating a single polarizer. Gibbons *et al.* constructed a cell in which one surface is coated with polyimide and the other with the dye-doped polyimide, and the plates arranged with rubbing directions parallel. The liquid crystal is then uniform and behaves optically as a birefringent slab (so that, for example, it would appear dark between crossed polarizers). Incident light from an argon-ion laser, polarized parallel to the initial direction of alignment, causes the liquid crystal molecules at the dye-doped polyimide surface to reorientate through 90°, forming a twisted-nematic structure which transmits light between crossed polarizers.

Clearly, this effect is due to the presence of the dye in the alignment layer. Similar effects have been observed in the bulk of azo-dye-doped monomeric[2] and side-chain polymeric[3] liquid crystals. Anderle *et al.*[4] suggest a mechanism for the photoinduced reorientation of the liquid crystal based on *trans–cis* isomerization of the azo-dye, changing the molecules' shape (see figure). Illuminating the dye with light of wavelength close to its absorption maximum induces the *trans–cis* reaction, with highest reaction probability for molecules parallel to the plane of polarization of the incident light because of the anisotropy of the dye's absorbance. The thermally excited reverse reaction, *cis–trans*, may occur by either of two routes but the final state is most likely to be perpendicular to the polarization of the incident light because of the continued excitation of the molecules in that direction.



Rotation of the optical axis of an azobenzene dye by photoinduced *trans–cis* isomerization (*a* → *b*). The thermally induced reverse reaction (*cis–trans*) is more likely to progress to orientation *c* than to orientation *a* if the optical axis of *a* is parallel to the polarization of the incident light.

A liquid crystal mixed with the dye will also become reoriented under these circumstances, owing to the molecular interaction between the dye and the liquid crystal, and therefore the optical axis of the sample will rotate to be perpendicular to the incident polarized light. Eich and Wendorff[5] have begun to demonstrate the considerable potential for these effects in liquid crystal polymers as a medium of eraseable optical storage, producing diffraction gratings and holograms. The possibility of real-time holography for display purposes in monomeric liquid crystals[2] is however limited by the slow response of the reorientation process.

Photoinduced isomerism may also lead to the surface alignment effects described by Gibbons *et al.* Two mechanisms have been proposed for the alignment of liquid crystals on rubbed polymers. One involves a long-range interaction due to surface microgrooves caused by the rubbing. The elastic energy of the liquid crystal is then minimized if the molecules lie along the grooves. Alternatively, the alignment mechanism may be a short range interaction between the liquid crystal molecules and the polymer surfactant. It is possible to distinguish between these mechanisms through measurement of the optical second-harmonic generation (SHG, a frequency-doubling effect) of the noncentrosymmetric monolayer at a surface[5]. The liquid-crystal/rubbed-polymer interface has been studied using this powerful technique by Chen *et al.*[6], who show, from the symmetry and polarization dependence of the SHG signal, a preferential, polar orientation of the surface layer of crystal along the rubbing direction of polyimide. Other alignment layers do not show such short-range, polar interaction and it is presumed that the alignment mechanism is due solely to the longer range elastic forces in the liquid crystal bulk. Although a dyed polyimide surfactant has not been studied by this method, the results of Gibbons *et al.* indicate that the liquid crystal molecules interact even more strongly with the dye than the polyimide.

Interesting new uses of liquid crystals may be emerging that take advantage of the fine, adaptive control of the orientation of liquid crystals using light instead of the conventional fixed electrode structures. But the mechanisms involved must be understood more fully in order to optimize the efficiency, response times and optical characteristics needed for practical devices. □

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RÉSUMÉ

Twinkle, twinkle

GRAVITATIONAL lensing may assist those searching for brown dwarfs, substellar objects that could account for much of the mass of our Galaxy. Because they are too small to 'burn' by nuclear fusion, brown dwarfs cannot be seen by their starlight, which is why they remain merely hypothetical. B. Paczyński reminds readers of the *Astrophysical Journal* (**371**, L63–L68; 1991) that the central bulge of our Galaxy is dense with slowly moving stars, and that as they pass behind the nearer stars in the galactic disk, gravity will focus their light to give us a brightened image. He points out that brown dwarfs could also focus background stars, though the brightening would not last so long (3–20 days instead of 1–4 weeks). In work in the press with *Astrophysical Journal*, Paczyński and S. Mao show that the same principle could be used to discover binary stars and remote planetary systems.

Bone hard

BONES are found far more often than soft tissue at archaeological sites, and offer a potentially huge harvest of DNA for the polymerase chain reaction — but only if the spectre of contamination can be exorcized. Hence the apparently inexhaustible capacity for taking pains shown by E. Hagelberg and J. B. Clegg in the isolation of mitochondrial DNA from a variety of bone samples (*Proc. R. Soc.* B**244**, 45–50; 1991). A pig bone from the larder of the *Mary Rose*, the ill-fated, sixteenth-century flagship of the English navy, for example, yielded DNA that is unequivocally porcine. Extracting DNA from bone, then, is not a problem, and the researchers recommend a stringent protocol to minimize spurious results from the exogenous DNA invariably associated with an archaeological sample.

Change for the worse

STUDIES reported by K. Hsaio *et al.* strengthen the case that damaged prion protein is at the root of several neurological diseases (*New Engl. J. Med.* **324**, 1091–1097; 1991). Some communities have an unusually high incidence of Creutzfeldt–Jakob disease, one such disorder, which has prompted several explanations of its cause, including the suggestion that consumption of the Mediterranean delicacy of sheep's eyes and brains is responsible. Hsaio and colleagues now confirm that an excess of the disease among Libyan Jews is more a result of genetics than of culinary taste: the disease occurs consistently in people in which a single amino-acid change has occurred within the prion protein, and the inherited genetic mutation seems to show complete phenotypic dominance in this population. The protein's normal function remains unknown, however.

GENE TRANSCRIPTION

TFIIB or not TFIIB?

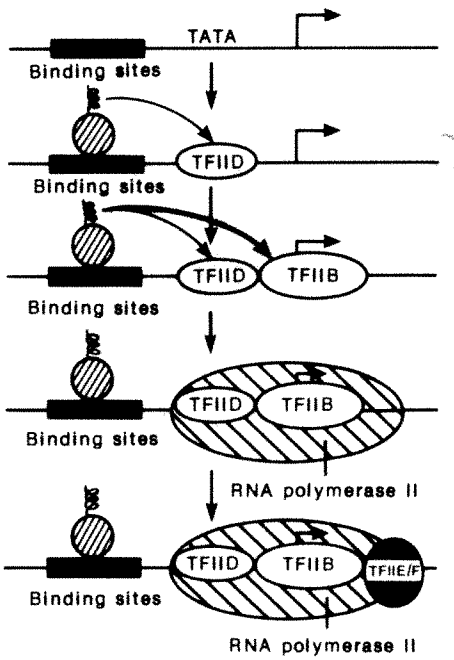
Phillip A. Sharp

LITTLE has been established about the mechanism by which sequence-specific DNA transcription factors stimulate initiation of transcription by RNA polymerase II (B). Two series of experiments now offer hope that this record will change. First, it is likely that transcriptional activation *in vitro* may be detectable only if the basal transcription reaction is repressed by chromatin, or more specifically by components of chromatin such as histone H1 (refs 1 and 2). Second, the major activation step in initiating transcription *in vitro* is probably not, as previously proposed, the template binding of the TFIID (TATA binding) factor, but the subsequent binding of the TFIIB factor to the TFIID–template complex[3]. This step forms the platform for the binding of RNA polymerase II and the TFIIE/F factor.

General transcription factors activate a basal reaction at the promoter site resulting in the positioning of the polymerase, and initiation. A distressing number of factors have been described as being important in this process. The simplest scheme is based on the assembly of template complexes which can be resolved by electrophoresis in native gels (see figure)[4]. The hallmark of this scheme is the ordered addition of factors where TFIID binding permits the association of TFIIA and TFIIB. Only after binding of TFIIB does the polymerase associate and the binding of polymerase is necessary for the association of TFIIE/F. This fraction is probably a mixture of two activities where the TFIIF component remains associated with the polymerase complex. Ideally, the rate of initiation of transcription reflects the rate-limited step in the assembly of this basal reaction.

Several sequence-specific transcription factors can bind to sites both proximal and distal to promoters and stimulate the rate of initiation of transcription. Because initiation requires the interaction of a number of basal factors with the TATA sequence and the polymerase, transcription factors are thought to increase the rate of the basal reaction. These factors possess one or more activation signals which, in theory, should promote the efficiency of binding or function of one or more of the basal factors that are rate-limiting for initiation by polymerase. The activation signals of transcription factors have been imprecisely defined as acidic, or proline- or glutamine-rich. Acidic protein domains that activate transcription possess a secondary structure, perhaps α-helical, and are apparently universally active in all organisms[5,6]. The acid-rich signal of the VP-16 protein of herpes simplex virus (HSV) has become the gold standard and has been analysed by mutations that change single amino acids. Both the acidic character and the conformation of this domain are important. Illustrative of the importance of conformation is that a single phenylalanine-to-proline alteration in the 78-amino-acid domain inactivates the signal[7]. Given the apparent simplicity and universality of the acidic activation signal, it was possible that a single universal step was accelerated by its activity.

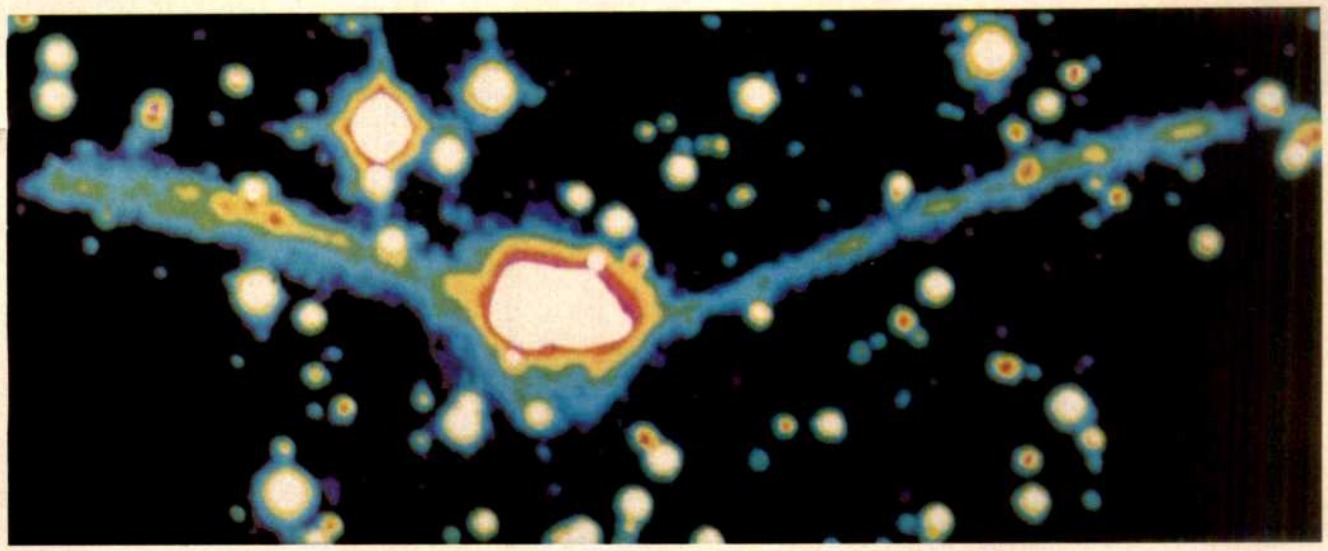
Disappointingly, addition of transcription factors with activation domains to reactions containing highly purified basal transcription factors results in only a modest 2–5-fold



A model for how an acidic activator stimulates transcription. (Adapted from refs 3 and 4.)

stimulation of transcription — not the 100-fold stimulation observed *in vivo*. Interestingly, addition of the same transcription factors to a reaction containing a total nuclear extract could stimulate transcription 100-fold. The difference between these two results has been interpreted to mean that there is a coactivator that mediates stimulation of the basal reaction by the transcription factor (for review see ref. 8), the hypothetical co-activator being missing from the reconstituted reaction.

Alternatively, a change in the nature of the rate-limited step in initiation of transcription could explain the difference. For example, the association of a basal factor might be efficient, and thus not rate limiting, in a reaction reconstituted by purified basal factors, but inefficient, and thus rate limiting, in a reaction formed with a total nuclear extract. The stimulation by a transcription factor which promotes the binding of this basal factor will be much greater in the reaction containing the total extract. Comparison of the efficiency of transcriptional activation *in vitro* in the presence or absence of chromatin



THE SUPERANTENNAE is the apt name given to this extraordinary object 250 megaparsecs (800 million light years) from our Galaxy. The object is the product of the collision, a billion (10^9) years ago, between two giant galaxies. Identified last year by J. Melnick and I. F. Mirabel (*Astr. Astrophys.* **231**, L19–L22; 1990), it is now shown by Mirabel and colleagues (*Astr. Astrophys.* **243**, 367–372; 1991) to be a massively scaled-up version of the more familiar Antennae, after which it is named. From tip to tip, the tails extend across 350 kiloparsecs (our Galaxy has a diameter of 30 kpc). The tails are the result of the tidal interactions between the two rotating galaxies, and are ribbon-like strips of gas pulled from the progenitors' original peripheral disks. The bright knots along them are regions where hydrogen gas has condensed to trigger new outbursts of star formation. The apparently equal spacing between the knots is a puzzle that may be solved by further observations using the European Southern Observatory's 3.6-metre telescope later this month. Beyond the tip of the southern tail (right-hand end) seems to be a knot as large as a dwarf galaxy. Mirabel comments that our Galaxy and our nearest neighbour, Andromeda, (comparable to the merging galaxies in the Superantennae) are approaching one another rapidly; but it will be several billion years before we know if we are to suffer a similar fate.

proteins suggests that the presence of components of chromatin may change the rate-limited step in initiation, and thus make the reaction more responsive to activator signals.

Workman and colleagues[1] have shown that reconstitution of chromatin on a template *in vitro* strongly suppresses the subsequent activity of the template for transcription with basal factors. However, if the reconstitution of chromatin is carried out in the presence of a transcription factor possessing an acidic activation signal, the basal reaction is efficient following addition of factors TFIID, TFIIB, polymerase II and TFIIE/F. In the last protocol, there was a 50-fold increase in transcription over the suppressed basal levels, dependent upon the presence of the transcription factor. The acidic domain of the transcription factor is essential for this activation of transcription following reconstitution of chromatin: if template is reconstituted in the presence of a mutant factor consisting of a sequence-specific DNA-binding domain, lacking its acidic activation domain, the template is poorly used by polymerase. Interestingly, the activation domain is not required for the binding of TFIID in the presence of the chromatin.

The histone protein H1 which binds to the linker DNA between nucleosomes is more prevalent on chromatin of transcriptionally inactive genes than on transcriptionally active genes; in fact, addition of H1 to chromatin containing 5S RNA genes inhibits transcription of these genes by added RNA polymerase III and factors[9]. The work described by Kadonago and colleagues[2] suggests that inhibition of transcription by histone H1 may be the specific aspect of chromatin responsible for suppression of transcription. Again, transcription of a template in a nuclear extract depleted of histone H1 was repressed by addition of purified H1. If the template contained binding sites for the factor, addition of a transcription factor possessing an acidic activation signal prevented repression. Thus in the presence of H1 the factors activated transcription more than 20-fold, a similar stimulation to that observed *in vivo*.

A most startling result from Lin and Green[3] suggests that the acidic activator functions by facilitating the association of the TFIIB factor with the TFIID–template complex. This insight arose from a novel experimental protocol, in which the template DNA was bound, by biotin modification, to a matrix of streptavidin. This permitted the rapid isolation of template-associated complexes and determination of the particular step in the figure that was accelerated by the presence of a transcription factor. Binding of the TFIID factor to the template was not increased by the acidic activator of the transcription factor. Surprisingly, association of TFIIB with the template–TFIID complex was highly dependent upon the presence of the transcription factor. The polymerase II and TFIIE/F components associated readily following the binding of TFIIB.

How does an acidic domain potentiate the binding of TFIIB with the template? The answer is probably by direct association of the acidic domain with TFIIB. When nuclear extracts are chromatographed over a column matrix containing an immobilized form of a protein related to VP-16, TFIIB activity is specifically retained. This association is dependent upon the integrity of the acidic domain in that the change of a phenylalanine to proline inactivates the binding of TFIIB. The finding of an interaction of TFIIB with the acidic activator domain and not TFIID appears to conflict with the earlier results of Stringer and coworkers[10], who reported the binding of TFIID and VP-16 using a similar assay. But Lin and Green[2] found that the TFIID/VP-16 association is much more sensitive to the concentration of counter ion than the TFIIB/VP-16 association. Thus, the picture emerges of a transcription factor located in a promoter or enhancer stabilizing the association of TFIIB, and perhaps more weakly TFIID, in the basal initiation complex.

From all these results one can predict that the presence of chromatin or histone H1 makes the initiation process highly dependent upon the step promoted by the acidic signal — that is, the binding of TFIIB. This follows from the facts that (1) the presence of chromatin components suppresses the basal reaction, thereby increasing the enhancement produced by activators; and (2) the acidic activator domain of these activators recruits TFIIB to the initiation complex. The simplest explanation for this is to suppose that the chromatin components obstruct the binding of TFIIB, making it the rate-limiting step in the process and thus increasing the dependence of the initiation reaction on the

presence of an activator domain which promotes the step. This suggests that the addition of a component that binds to promoter DNA in a nonspecific fashion (that is, chromatin proteins) could under some conditions satisfy some of the assay properties suggested previously for coactivators. In a mechanistic sense, little is known about the kinetics of TFIIB binding to the TFIID–template complex. The specific footprint generated by the binding of TFIIB over the initiation site is not pronounced and is confined to one strand of the template[4], so TFIIB may not be strongly bound to the template in the absence of an activator domain.

Final answers to most of the questions addressed by these experiments await the purification and analysis of the specific proteins that constitute TFIIB and the other basal components. The most purified preparations of TFIIB contain a protein of relative molecular mass 32,000, and specific antiserum has been prepared[11] which reacts with protein in the template TFIID–TFIIB complex. It is highly likely that a complementary DNA encoding this factor will be available shortly, and more refined studies of the interaction of the protein with the acidic activator and TFIID will then be possible. □

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NEUROBIOLOGY

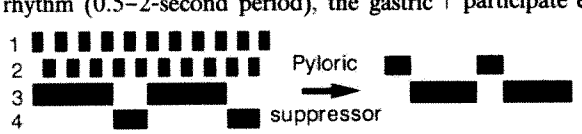
A new act to swallow

Eve Marder

On page 60 of this issue[1], Meyrand and colleagues demonstrate that the same neurons can be part of several different functional networks, some of which may only exist operationally for a short time if the behaviour they control is not continuous. This contrasts with the view that each neuron has a specific and unique role in sensory processing or motor function; for example, one might imagine that the networks that generate walking are activated during walking, but when the animal is not walking these neurons are not used. The view of the organization of the nervous system that Meyrand *et al.* now provide shows surprising similarity to one seen in neural network theory, in which individual elements can be used to construct many different functional networks.

Meyrand *et al.* studied the neural networks of the crustacean stomatogastric nervous system[2], which consists of four ganglia: the stomatogastric ganglion with 30 neurons, the oesophageal ganglion of 18 neurons, and the two commissural ganglia, each with several hundred neurons. When removed from the animal, the stomatogastric system retains the ability to generate several different rhythmic motor patterns. These include the pyloric rhythm (0.5–2-second period), the gastric and oesophageal rhythms (each of 5–10-second period), the cardiac-sac rhythm (30–120-second period), and a swallowing pattern described for the first time in the new work[1]. The cellular properties and circuit interactions responsible for generating the pyloric and gastric rhythms are relatively well understood because the neurons involved are relatively few in number, and are large and uniquely identifiable regardless of their firing patterns, and because the motor neurons themselves are important components of the pattern-generating networks.

For many years biologists have treated the stomatogastric nervous system as if it were divided into neat, almost independent, subsystems, each responsible for the generation of a different motor pattern. In this view, neurons participate in only one motor pattern, and are classified as 'pyloric' or 'gastric' on the basis of their anatomical projections and firing patterns. But then Hooper and Moulins[3] showed that the ventricular dilator neuron could switch its firing pattern between the pyloric and the cardiac-sac rhythm. And Weimann *et al.*[4] demonstrated that many neurons previously thought to participate exclusively in the generation of the pyloric or gastric rhythms could fire in time with either rhythm. Furthermore, Dickinson *et al.*[5] showed that a peptide (red pigment concentrating hormone) could elicit a novel rhythm in which gastric and cardiac-sac neurons were coordinately active. Meyrand *et al.* now show that, when the animal swallows, a pair of pyloric suppressor neurons are activated which override the synaptic and cellular events responsible for the generation of the pyloric, gastric and oesophageal rhythms, and which recruit some of the elements of these networks to make a new network to generate the swallowing motor patterns.



Block diagrams showing the activity of different neural elements. Traces 1 and 2, pyloric elements; traces 3 and 4, gastric elements. Under control conditions (left) the faster pyloric and slower gastric rhythms occur simultaneously. Pyloric suppressor activity evokes a novel pattern using elements of both the fast and slow rhythms. Other elements become inactive (traces 1 and 4).

The pyloric suppressor neurons can be activated by stimulating sensory pathways. The neurons then act at many sites in the stomatogastric nervous system: they drive motor neurons that control the opening of the oesophageal valve for swallowing; strongly inhibit many of the neurons of the pyloric and gastric networks; and strongly excite other neurons of the pyloric, gastric and oesophageal networks. The net result is that bursts of action potentials in the pyloric suppressor neurons evoke a new motor pattern in which neurons of the pyloric, gastric and oesophageal networks fire coordinately with each other and with the neurons that operate the oesophageal valve (see figure).

It is important to remember that the stomatogastric nervous system is different from many in that the motor neurons themselves are intrinsic components of the central pattern-generating circuits. Therefore, when an identified stomatogastric ganglion motor neuron alters its activity pattern, the behaviourally relevant output and the effect of neurons that shape this output are seen simultaneously. So the new studies show that the same neuron can participate in several distinct neural networks. They also demonstrate that operational networks can be formed by synaptic and modulatory inputs when needed, but that their elements can be freed for other tasks at other times.

Some may be tempted to argue that the kinds of circuit reconfigurations that can be seen in invertebrates are not necessary in the vertebrate nervous system, with its vastly larger number of neurons. I remember when a similar argument was made about the ability of invertebrate neurons to generate bursting pacemaker and plateau potentials. Now we know that vertebrate neurons can display as rich a range of membrane properties as has been long known for invertebrate neurons. I await the time when workers on vertebrate preparations can reliably identify neurons independently of their firing patterns. We will then see the extent to which neurons in the vertebrate nervous system are used as elements in many different functional circuits. □

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ARCHAEOLOGY

Another (and armless) army

Paul G. Bahn

ANOTHER terracotta army has been discovered in Shaanxi Province, China, in the same region as the famous one found in 1974. The new army, now described by W. Zhaolin and M. Hong (*Archéologia* **265**, 4–5; 1991), is much smaller in stature, but looks to be far larger in numbers.

The discovery 17 years ago, by a farmer digging a well, of the life-size terracotta army of China's first emperor Qin Shihuangdi led eventually to this "eighth wonder of the world" becoming an attraction for tourists, who come in droves to see the 7,000 clay warriors still standing in their vault, now protected beneath an enormous hangar. Such has been the popularity of this archaeological marvel that plans were made to build a new airport, which will require a new 19-km motorway to link it with the city of Xian.

It was in the course of constructing the motorway in March last year that the new find was made. Four necropolises of the Han (206 BC–AD 220) and Tang (AD 618–907) dynasties had already been encountered in the vicinity. Tests were made in a wheatfield located 300 m south of the tomb of the wife of the emperor Liu Qi, who was known during his reign as Jing and who lived from 188 to 144 BC; he and his wife were buried at Yangling, 22 km northwest of Xian.

At a depth of 6.5 m, a small orange potsherd was found, which was soon identified as the shoulder of a statue. The whole field was then prospected, and the Archaeological Institute of Shaanxi Province sent in a team of specialists in the study of Han tombs, led by Wang Xueli, chief excavator of the famous terracotta army.

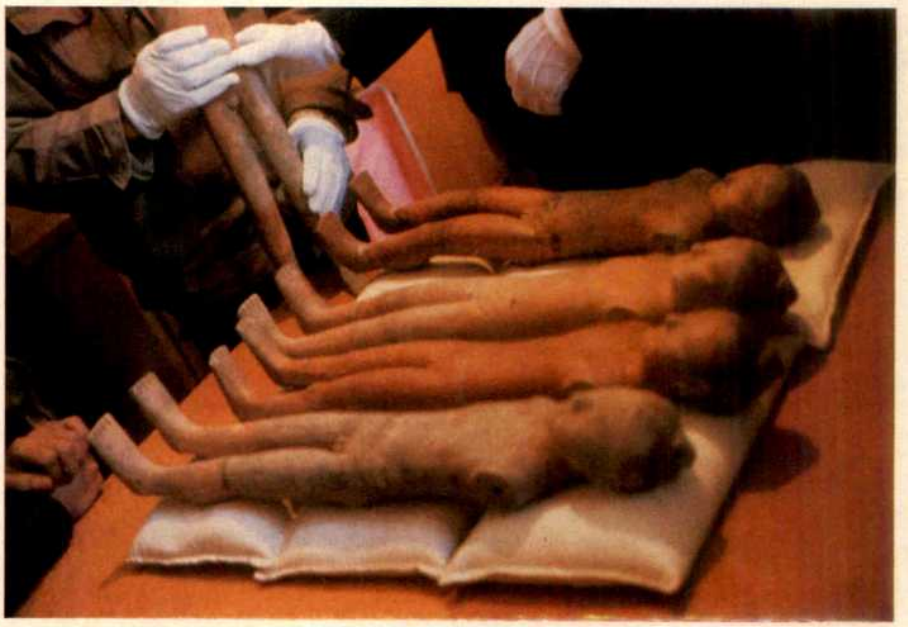
The new site proved to cover 96,000 m^2, the size of twelve soccer pitches. It contains an army of terracotta figures, associated with the two tombs, and housed in 24 vaults about 20 m apart and in 14 rows aligned north–south. The vaults are 4–10 m wide, and 25–291 m in length. They are therefore smaller than the Qin dynasty pits, but they cover an area about five times as large. Their different sizes may correspond to those of the army units they contain (one contains mostly cavalry, another may be the headquarters).

All the human statues are of naked men, with no arms; they are about 50 cm high. The whole body is painted an orange–red colour, with hair, eyebrows, beard and eyes coloured black. Their clothes, assumed to have been of linen or silk, have disintegrated. There are different theories about the arms. Some researchers believe they may have been held on with rods of precious metal, which were subsequently stolen. Others think it likely that they had movable wooden arms, which have disintegrated like the clothing.

The figures have graceful forms and delicately sculptured faces. Each one is different from his fellows in age and in facial expression, which ranges from severe to smiling. In their remarkable realism they resemble the life-size figures which are about a century older. But it is the differences that are the most striking: not only are the Qin figures life-size, but their arms and their uniforms form an integral part of the statues. Also, the Qin figures are hollow, whereas the new discoveries are solid.

The new army is accompanied by weapons of copper and iron, arrowheads, spears, swords, chisels and agricultural tools, carts, jewellery and coins — all of them, like the men and horses, one third life-size.

More than 300 statues have been uncovered so far, but it is certain that thousands remain buried; estimates of the total vary from 10,000 to a million, and excavation will continue for two to three years. Meanwhile, the motorway project goes ahead, though now a bridge, 320 m long, will be constructed over the site, and an underground museum is being planned to enable the inevitable tourists to see the new army. □



Minute men of China's new model army (picture courtesy *Archéologia*).

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REPRODUCTIVE BIOLOGY

Do sperm find eggs attractive?

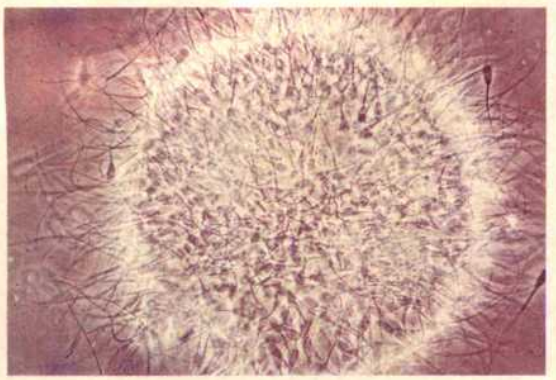
John Aitken

STUDIES described by D. Ralt and colleagues in *Proceedings of the National Academy of Sciences*[1] suggest that the conventional wisdom about how male and female gametes make contact in the reproductive system of the human female is wrong. The authors provide evidence for the existence of a chemotactic factor that attracts spermatozoa to the egg, thereby opening up an entirely new perspective in reproductive biology.

In the past, the best evidence for sperm chemotaxis has come from invertebrate species, such as sea urchins, that engage in external fertilization. These species shed their gametes into the ocean, necessitating the evolution of intricate species-specific mechanisms to ensure that the spermatozoa not only find eggs, but eggs from the right species. By contrast, spermatozoa in species exhibiting internal fertilization, in which the sperm are released into the confines of the female reproductive tract, were thought to find their quarry by behaving like Scottish salmon heading up-river towards their spawning grounds.

This analogy was based on the fact that the only form of orientation recognized in human spermatozoa was rheotaxis, in which the male gametes swim against the current in a moving body of medium. Because the flow of fluid in the female reproductive tract is outward, the spermatozoa inevitably move upstream, in the direction of the ampullary region of the fallopian tubes, where fertilization occurs. Initial contact between the male and female gametes was thought to be the consequence of a chance collision, followed by a highly specific, cell-recognition event that resulted in the tight adhesion of the spermatozoa to an acellular membrane surrounding the egg, known as the zona pellucida.

Ralt and colleagues now propose that the fluid that bathes the oocyte during its dif-



Spermatozoa–egg interaction during mammalian fertilization. The results from Ralt *et al.*, discussed here, imply that the initial contact between human gametes is the result of sperm being attracted by chemotactic factors.

ferentiation within the ovarian follicle (follicular fluid) contains chemotactic factors for human spermatozoa. Their experiments involved the use of two chambers separated by an 8-μm polycarbonate filter. Spermatozoa were placed in the lower compartment and their ability to migrate through the filters and accumulate in samples of diluted follicular fluid in the upper compartment was assessed. The controls consisted of parallel incubations in which the upper wells contained either culture medium or blood serum, or incubations in which follicular fluid was added to both the upper and lower chambers.

The authors observed a clear accumulation of spermatozoa in the upper chambers in the presence of follicular fluid diluted 1:5,000 to 1:10,000, but no such accumulation in the controls or in dilutions of follicular fluids outside this range. That the accumulation of spermatozoa in the presence of diluted follicular fluid was due to chemotaxis and not a number of alternative explanations was confirmed by looking at the trajectories of human spermatozoa following the microinjection of follicular fluid into the sperm suspensions. Some of the spermatozoa changed their swimming direction towards the site of the fluid injection. Exactly the same behaviour was observed when egg-conditioned culture medium was microinjected into suspensions of human spermatozoa.

One of the intriguing features of the experiments is the considerable variability exhibited by follicular fluid specimens in their ability to influence the swimming trajectories of the spermatozoa. Indeed, 50 per cent of the samples possessed no activity whatsoever. Such variability would cast severe doubt on the authors' conclusions but for a remarkable correlation between the expression of chemotactic activity by the follicular fluid and the ability of the egg from the same follicle to undergo fertilization *in vitro*. In a blind analysis of 62 follicular fluids from 40 patients there was a highly significant difference in the level of chemotactic activity in follicular fluid samples associated with eggs that did fertilize *in vitro* compared with those that did not.

If confirmed, these observations will have very many ramifications. The existence of chemotactic factors that accumulate during preovular development would provide a means of monitoring the maturational status of oocytes aspirated from the ovary during therapeutic procedures such as *in vitro* fertilization and gamete intrafallopian transfer. Assessment of the chemotactic activity expressed by follicular fluids would be an effective way of monitoring the ability of different ovulation-induction regimes to generate populations of mature preovulatory follicles, and it would also help in identifying causes of female infertility associated with a failure to generate the factor concerned. Conversely, the observations of Ralt *et al.* imply that receptor sites for the putative factor must be present on the surface of human spermatozoa, the absence of which could be involved in male infertility. Finally, if the chemotactic factor has a part in mediating the union of human gametes at fertilization, the potential for developing radically new approaches to contraception is considerable.

But, as Ralt *et al.* point out, theirs are but preliminary studies which pose as many questions as they answer. What, for instance, is the chemotactic factor? The major follicular steroid, progesterone, is one possibility, because human spermatozoa respond to its presence by generating calcium transients, indicating the presence of some form of progesterone-receptor mechanism at the sperm surface[2]. Ralt *et al.* showed however that chemotactic activity was not correlated with the concentrations of progesterone or oestrogen in the follicular fluids. Other candidates include peptides structurally related to the neutrophil chemoattractant, *N*-formyl-methionyl-leucyl-phenylalanine: receptors for this peptide on the human sperm surface have been described, and a claim made that it induces chemotactic behaviour in human spermatozoa[3].

In addition to the physico-chemical nature of the putative chemotactic factor, the stage of oocyte maturation at which it is elaborated, the ontogeny, distribution and structure of the complementary sperm receptor sites and the intracellular mechanisms responsible for the change in flagellar beat characteristics responsible for chemotaxis, all require investigation. Despite the tentative nature of the new work, the biological and clinical implications are such that gamete biologists are unlikely to get much rest in the coming months. □

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DAEDALUS

Washing the wind

Air pollution is very hard to control. Once something has escaped into the air, you can't get it back. And yet the various layers of the atmosphere mix only slowly. A lot of smog and pollutant gases linger near ground level for quite a while to plague us all. Some purifying device which made contact with a lot of ground-level air could greatly improve our environment.

The ideal device, says Daedalus, is that epitome of greenery, the windmill. To maximize its efficiency, the blades of a windmill must make contact with as much of the wind as possible. If the blades were coated with a catalyst that oxidized the smog or pollutant on contact, they would purify the air as it passed through.

At first Daedalus thought of adapting the catalysts used to clean up car exhausts. These, however, only work near red heat — a worrying complication in a windmill. Photochemical catalysts based on titanium dioxide seem a better option. In the presence of light and moisture at room temperature, this material converts hydroxide ions to hydroxyl radicals — which oxidize organics rapidly and ferociously. A windmill coated with a hygroscopic titanium dioxide photocatalyst would only work in the daytime. But it would then convert the thickest smog to country-fresh air, and waft it healthily downwind. Under the right conditions, a well sited windmill farm could deliver both power and clean air to the fortunate suburb sheltering in its lee.

Even better, says Daedalus, the oxidation of the air pollutants will help to drive the windmill. Most pollutants, such as carbon monoxide, methane and other hydrocarbons and solvent vapours, liberate a lot of heat when they are oxidized. The product molecules will spring off the catalytic surface with greatly increased thermal velocity, imparting significant thrust to the surface. If the windmill blade is selectively coated with catalyst on its trailing edge, this thrust will help to drive the blade.

To test this elegant notion, DREADCO engineers are painting catalytic surfaces on fans which have to pump very polluted air anyway, like the exhaust fans of road-tunnels, paint shops and chemical works. They should deliver cleaner air for less power — indeed in really dirty air they may even run spontaneously. Once the concept has been proven, Daedalus will offer his catalytic coating technology to the designers of windmills and industrial fans. Even cars, those arch-polluters of our environment, could make amends with catalytically coated cooling fans, while catalytic propeller-driven aircraft could make a token contribution to purifying the upper air. Indeed, says Daedalus, a catalytic coating on their under wing surfaces should not only clean the air they fly on, but slightly increase their lift. David Jones

Designing better solar cookers

SIR — In their Commentary on solar cooking[1], Kammen and Lankford gave pride of place to the box-type cooker. But this design suffers from several drawbacks, not least that such cookers require cooking to be done in direct sunlight and during the middle of the day, and that they lack storage facilities and have no provision for frying.

In a bid to overcome some of these problems, a cooker with fresnel lens was designed[2]. A large fresnel lens is used to heat a container surrounded by an annular cavity filled with ammoniated salts of magnesium chloride and calcium chloride. Heat is stored chemically in these compounds and is released on demand at 300 °C.

Another system that has storage but is less expensive than the above, uses heat pipes for parabolic as well as flat-plate collectors[3]. In this arrangement, an evaporator with minimum shading effect is placed at the focus of a solar collector. The energy reaching the evaporator is conveyed rapidly to the condenser end of the heat pipe. The condenser, in the form of a cooking chamber, is located in the shade or inside the kitchen. As the shaded cooking chamber is well insulated, pot losses associated with wind will not arise. This system uses energy from sunshine and will not allow reverse circulation losses, due to its diode-like operation[4]. Because of the unique feature of isothermal operation of heat pipes, temperatures on a flat-plate collector equipped with a heat pipe will be forced to follow the temperatures in the condenser section.

A more recent addition to the solar cooker family is that developed by Mills and his group at the University of Sydney[5,6]. This cooker can do practically anything a gas or electric stove can, and because it includes a small pressurized water vessel, it can also heat water or power a refrigerator. It can make two large family meals on sunny days and can easily be supplemented with conventional fuel on cloudy days. It has no moving parts, makes use of common materials and can fry, bake, boil or steam food. Its hot plate can be placed indoors, and heat storage allows cooking after sundown.

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KAMMEN AND LANKFORD REPLY — Jagadeesh's letter is similar to several that we have received in response to our article[1]. It describes technically sophisticated designs for solar cookers that will achieve higher temperatures than our simple box-cooker. The question is whether they will be used or not.

Not only are the designs technically complex and rather expensive, but one of them uses magnesium chloride and calcium chloride battery salts that could be harmful to livestock or children if the batteries were dumped or broken and the salts consumed.

We strongly support the notion that a diversity of solar and other renewable technology systems is necessary to support energy self-sufficient development, but we feel that Jagadeesh has missed the essential point concerning the ovens. We have found that when the end user (nearly always a woman) makes her own oven, her interest in using it is very high. If someone else makes and donates the oven or significant components (such as ammoniated salt batteries, condensers or pressurized water vessels), her interest is substantially less. For this reason we believe that until solar cooking equipment has become commonplace a design that can be made locally is essential for widespread acceptance of this technology[7].

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Olbers' paradox

SIR — At least in a mathematical sense there is yet another solution to Olbers' paradox[1]: fractals[2]. Fractal structures like Menger's sponge can have infinite surface area but zero volume and show a self-similar, scaling-independent distribution of their points. Suppose galaxies, clusters and higher order superclusters of galaxies (or, more exactly, the total mass energy including dark matter) would be distributed in such or a similar fractal fashion. Due to the fractal structure bigger volumes have to contain a relatively lower amount of matter and energy. The energy (and thus also radiation) content per volume falls below any given positive boundary only if a sufficiently large total volume is looked at. Even if it should be static, unlimited or eternal, the sky in such a fractal universe remains dark at night. And so, too, does the sky in our own expanding, finite-age Universe, but what about its large-scale structure[3]; are there some underlying fractal properties?

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Prion infection

SIR — The finding that the chaperonin heat shock protein 60 (hsp60) catalyses the assembly of further hsp60 molecules[1] lends weight to the fascinating possibility[2] that prion infection might be due to self-perpetuating protein-directed changes in topology. Although, as both Carlson *et al*[2] and Weissman[3] comment, such replicatory topology alteration is unprecedented as a mechanism of infection, the case of hsp60 illustrates that it is not without precedent as a means of protein topology change. The similarity between the suggested mechanism of prion infection and hsp60 activity is perhaps strong enough to suggest that prion protein could be a rogue chaperonin. Further, the hypothesized topology alteration of hsp60 and prion protein suggests that that which replicates need not be nucleic acid.

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Neotropical plant diversity

SIR — We believe that the overwhelming emphasis on lowland forests with regard to tropical conservation and deforestation has diverted attention from certain patterns of neotropical plant species diversity (see for example, refs 1 and 2). Specifically, the northern Andes, a relatively small and highly deforested region, may have a flora as rich or richer than that of the much larger, mostly intact Amazon basin. Areas of the world that contain the greatest diversity of species and face the most immediate threat of destruction should be given priority for conservation and study.

In most groups of organisms, diversity increases from high to low latitudes and from high to low elevations. The lowland Amazon rainforest has become a popular symbol of a beleaguered stronghold containing millions of species, most of them unknown to science. But the Amazon basin is far from the richest region in the neotropics when plants are considered. Other, smaller regions, such as the northern Andes, Central America and the Atlantic coastal forest of Brazil, are comparatively as rich or richer, and are almost completely deforested, whereas the Amazon forest is relatively intact (see table).

There is no published account of all neo-

DEFORESTATION AND DIVERSITY IN THE NEOTROPICS

Phytogeographic region*	Amazon basin	Northern Andes	Atlantic coastal forest of Brazil	Central America and Mexico
Surface area in sq km†	7,050,000	383,000	1,000,000	2,515,295
Area deforested (%)‡	8–11	90–95	>95	cs 60
Estimated total number of species ∫	30,000	40,000	10,000	19,000
Flora neotropica Sample ll	1,347	891	793	1,038
Gentry's sample ¶	1,829	1,454	1,288	1,934

* The four most diverse neotropical phytogeographic regions from Gentry[7]; elevational boundaries between regions are the 500 m contour.
†Surface area is plane surface and does not take into account topography.
‡ Figures from: Amazon basin (Brazil only)[8]; Northern Andes[9,10]; Atlantic coastal forest of Brazil[11]; Central America (excluding Mexico) from G. Hartshorn, personal communication.
∫ Figures from Gentry[7,9] and Myers[11]. Figure for Central America excludes Mexico.
ll Sample taken from 43 volumes of *Flora Neotropica* (flowering plants only) dealing with 5,331 species (that is about 6 per cent of estimated total of 90,000).
¶ Sample taken from Gentry[7].

tropical plants, and even the total number of species is unknown. Many botanists accept an estimated total of 90,000 species of flowering plants. There are some estimates of how this total may be distributed amongst the various neotropical phytogeographical regions. We have used these, as well as the results of two samples, to get an idea of the relative diversity of each region (see table).

The table shows that, for its size, the northern Andes is the most diverse region in the neotropics, and its forests have been progressively reduced for centuries. Although this region has a surface area only one twentieth that of the Amazon basin, it contains at least as many species of flowering plants. Among the bryophytes, 93 per cent of the approximately 900 species of moss known from Colombia are found in the Andean region, which accounts for only 20–25 per cent of the Colombian land surface; the remaining 7 per cent are restricted to the surrounding lowlands. Only 200–250 species of moss occur in the entire Amazon basin. Hepatics show similiar trends in Colombia, approximately 80 per cent of the species being restricted to the Andes[3]. Among pteridophytes, the Andean region contains approximately 1,500 species of fern and fern allies, whereas, in contrast, the Brazilian Amazon basin contains only 280 species[4]. The homosporous ferns are also particularly numerous in the Andes: approximately 1,000 species are recorded from Ecuador (300 of which are from the eastern lowlands). The figures are smaller for lowland neotropical countries, for example, 260 for Suriname and 220 for Brazilian Amazonia[5].

Much of the data in support of the diversity of lowland neotropical forest have come from recent inventory studies. Spectacular results have been recorded. For example, 300 species of tree with a diameter at breast height of at least 10 cm have been found in one hectare in Amazon Peru[6]. We do not question that there are many tree species in lowland neotropical forests, but our argument is for all plants and not just trees. By using the standard sampling technique that includes only woody plants with stem diameters equal to or more than 10 cm (usually in 1 hectare sites), most of the plants present are probably missed. Unfortunately, there is not, to our knowledge, any inventory of primary forest that takes account of all plant species, including bryophytes.

Our purpose here is to draw attention to the diverse, threatened flora of the northern Andes, which we feel attracts disproportionately little interest from biologists and conservationists. We do not understate the importance of the Amazon basin and its biota. But figures such as 8–11 per cent total deforestation in the Amazon basin pale in significance when one considers the northern Andean region.

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Is cold dark matter really dead?

SIR — Our article in *Nature*[1] and the accompanying News and Views[2] appear to have been widely interpreted as ruling out the existence of cold-dark-matter. Although the article makes it quite clear that it is the standard cold-dark-matter model that is questioned by our data, we are concerned that workers in other fields may not fully appreciate the distinction. The standard model is based on four premises: (1) that dark matter consists of weakly interacting 'cold' elementary particles; (2) that the Universe has critical density; (3) that the seed fluctuations from which cosmological structure grew are of the type predicted by the inflationary theory of the early Universe and (4) that the distribution of galaxies is related to the distribution of mass through a simple statistical prescription, the 'linear biasing model'.

The discrepancy between the size of the superclusters in our map of galaxies detected by the Infrared Astronomical Satellite (IRAS) and the predictions of the standard model imply that one or more of the model assumptions is incorrect. It does not automatically rule out the existence of cold dark matter. Indeed, in two separate studies[2,3], we have shown that the dynamics of the superclusters indicates that the Universe has a density very close to the critical value — as hypothesized in (2) above. Standard Big Bang nucleosynthesis limits the density of baryons to less than about 10 per cent of the critical value. Thus, our data from IRAS provide strong evidence that the Universe is dominated by non-baryonic dark matter.

A viable cold-dark-matter theory might grow out of the standard model through a more physical biasing prescription or alternative seed fluctuations, perhaps associated with cosmic strings or textures. Exploring these and other possibilities is one of the exciting challenges facing cosmologists today. Ultimately, however, the cold-dark-matter paradigm will stand or fall on the basis of the laboratory searches, now underway in the United Kingdom and elsewhere, for the elementary particles it postulates. We believe that these searches should continue to be energetically pursued.

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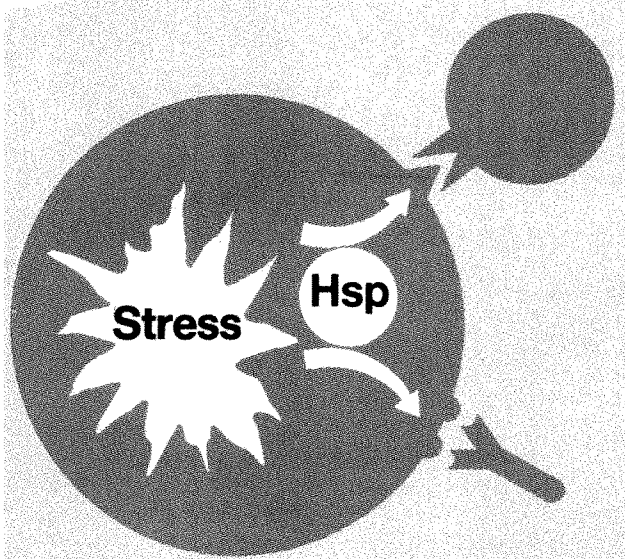

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Legislating for clean water

Eric Ashby

The Limits of Law: The Public Regulation of Private Pollution. By Peter C. Yeager. *Cambridge University Press: 1991. Pp.369. £35, $49.50.*

In the 1970s, the US Army Corps of Engineers was about to construct a $1.2 billion hydroelectric dam on the St John River in Maine. The river is not in a region of virgin wilderness. It is an area for potato farms and woodpulp mills. But on its steep banks, among spruce and alder, there grows a rare plant, the Furbish lousewort, named after the amateur botanist who discovered it. It is a modest plant, but under the Federal Endangered Species Act it is protected. Accordingly, in 1977 President Carter stopped further work on the dam until the future of the Furbish lousewort could be assured.

How did the US government, cradle of the military-industrial complex, come to pass a law like that, constraining our exploitation of nature, often much to our inconvenience? In *The Limits of Law*, Peter Yeager examines this question as it applies to laws to control industrial water pollution in the United States. He records in detail the growth of federal law to clean up rivers, culminating in the historic and euphoric *Federal Water Pollution Control Act Amendments* in 1972; an Act historic because it was passed by Congress in the teeth of a veto by President Nixon; euphoric because it aspired to make all rivers in the United States "fishable and swimmable" by 1985.

Yeager's detailed account is attached to a theme, namely that the drafting of anti-pollution laws, the regulations made under them, and the commitment to enforce them, were all limited by the bias of politicians, and self-interest of polluters, and latterly by the greening of public opinion. A subsidiary theme is that these limits were imposed under the influence of élites, especially large corporations, often at the expense of small business and the general public. As background to his essay, Yeager discusses how laws are made to regulate social activities like industry. He concludes that they are not the product of consensus between lawmakers and those they seek to regulate; they are the product of conflict between industrialists who want to exploit the environment and politicians who want to safeguard the interests of other voters as well as to keep the good will of industrialists. Participating on both sides of the conflict are technical experts, whose advice may set limits to the whole process, from drafting of the law to enforcement of the regulations. Supported by anecdotal evidence and some dubious regression coefficients, Yeager gives the impression that big industry has had more than its fair share in setting limits to cushion the effects of legislation, to make the wording of some laws 'symbolic', the regulations ambivalent, and the enforcement supine.

But the constraints that limit law making and its enforcement do themselves change. Thus, for a time industrialists called the tune, but when toxic chemicals got into the drinking water, consumers joined the conflict and influenced the limits more in their favour. The same law may then be reinterpreted by the officers in the field who have to enforce it. At this stage industrialists develop what Yeager quotes as 'corporate responsibility', taking a lead in pollution control to restore the confidence of the public.

Greenpeace

Discharge pipeline from a large pharmaceutical company.

All this is familiar to anyone concerned with environmental policy in Europe and Yeager does not make any fresh contribution to it. The interesting part of his book is the detailed account of the debates leading to the critical law passed in 1972 and its aftermath. Such an enterprising law needed an enterprising bureaucracy to administer it. That was to hand. The Environmental Protection Agency (EPA) had been set up in 1970. It had 6,000 employees and an initial budget of $1.4 billion. The first task of its Water Quality Office was to get data on pollution from thousands of industrial polluters and to decide how much each of them should be allowed to discharge. Industrialists then challenged the proposed consents. By 1973 there were still over 1,100 requests for hearings pending. In its initial enthusiasm the recommended to the Justice Department that lawsuits should be launched against 200 corporations. Meanwhile, there was confusion about the way controls should be achieved. The original recommendation was for the use of the Best Practicable Technology (BPT). But of course the word 'practicable' is a bonus for lawyers in court and there was pressure, when it was disclosed that carcinogens were among the pollutants, to abandon BPT for BAT — the Best Available Technology.

In the first flush of its activities the ugly words 'cost-benefit' were not audible in the corridors of EPA. But lobbying in Washington on the part of corporations alerted the Office of Management and Budget (OMB) to the hazards to industry of an environmental agency that was too zealous. It was suspected that OMB "often stalled the EPA's legislative proposals and testimony to Congress, giving advantages to industry . . ." and "might even be tampering with technical data" which supported EPA's case for stricter control. It was President Reagan who finally sabotaged EPA's strategy. Private sector costs were to take precedence over public-sector benefits. EPA's budget was cut. Its administrator was told to submit cost-benefit statements for all regulations. This amounted to deregulation. That was in 1981 and arguably it has handicapped American environmental policy ever since. In the end it has been the courts of law which have defended EPA against the erosion of its powers. But deregulation, too, has its limits and constraints. In the 1980s there was a massive legal action brought by a group of major corporations against the EPA, challenging its estimates of the cost of pollution control. They thought they were riding on the wave of deregulation. They were wrong: in April, 1989 the appellate court unanimously rejected their challenge, and upheld the EPA rules.

It is a reviewer's duty to issue a warning about *The Limits of Law*. Readers of books like this do not expect them to be written in polished prose. They are prepared to read, two or three times over, sentences conveying subtle and elusive ideas. But readers do expect straight reportage (which is the main material in this book) to be intelligible at first reading. Peter Yeager does not meet this expectation. His sentences sag under the sheer weight of abstract words. There are rich examples of what H. W. Fowler in *Modern English Usage* (Clarendon, 1965) called "abstractitis". It is a style of unparalleled viscidity. The effort of reading it can be compared to the effort of walking through a swamp in over-sized gum boots. It is a pity that the book is so exhausting to read, for Yeager has an interesting and important story to tell. □

Lord Eric Ashby is at 22 Eltisley Avenue, Cambridge CB3 9JG, UK.

In the eye of the beholder

J. Anthony Movshon

Vision: Coding and Efficiency. Edited by Colin Blakemore. *Cambridge University Press: 1991. Pp.448. £65, $120.*

In a scientific career spanning five decades, H. B. Barlow — one of the seminal figures in modern sensory physiology — has contributed extensively both to our understanding of visual processing and to the way in which we conceptualize the brain's representations of sensory information. *Vision: Coding and Efficiency* — a tribute to Barlow — derives from a symposium held in Cambridge in 1987 to celebrate his 65th birthday and his concurrent retirement from his Royal Society professorship, if not his retirement from science (he remains active today). Edited by Colin Blakemore, Barlow's first student, this handsomely produced book spans the extraordinary range of fields in which Barlow has made his presence felt.

The range of topics represented is both the strength and the weakness of the undertaking. Unlike many *festschriften* for lesser figures, which tend to collect too many papers devoted to a narrow subfield, this effort has a broad coverage of many areas of modern visual science, from fundamental optics, psychophysics and neurophysiology through conceptual models of image understanding. The result is almost the skeleton of a textbook of visual science. The only difficulty is that the coverage is so broadly based on the interests of those who have been associated with Barlow over the years that it is inevitably a bit uneven.

Nonetheless, there is much to treasure. The roster of authors is a distinguished one indeed, and a surprisingly large proportion have produced papers that are not simply minor revisions of their standard 'canned' review article. Notable among these are D. G. Pelli's lead chapter on quantum efficiency, which lays out an elegant framework (owing much to Barlow) for understanding the concept of statistical efficiency as applied to vision. D. Kersten's chapter is a brave effort to extend this kind of concept to higher-level processes of image understanding. Other papers on various aspects of spatial vision, light and dark adaptation, and colour vision, all add to a satisfying and coherent group of readings on the analysis of visual function by psychophysical techniques.

Coverage of visual neurobiology and development is a bit patchier. The book contains an intriguing mixture of papers on comparative topics (noteworthy and elegant are contributions by J. D. Pettigrew on stereopsis and by M. F. Land on compound eyes), on retinal function and organization (among them an appealing but stylistically dissonant piece on retinal heterogeneity by D. I. Vaney and A. A. Hughes), and on cortical processing (among them useful developmental papers by C. Blakemore and by R. C. Van Sluyters and his colleagues, and an extensive review of recent work on cortical architecture by S. M. Zeki). Because this part of the collection seeks to cover a vast and growing field, however, it leaves open rather more gaps than it fills.

Many of Barlow's most enduring contributions have been theoretical, and theory has its place here to. Again one might quibble at some unevenness and eccentricity; A. B. Watson's alarmingly crystalline concept of visual cortical processing stands out as a provocative effort in a slightly mundane set of chapters, as does Barlow's own contribution, a characteristically thought-provoking piece on the basis for visual after-effects.

It is a little hard to decide for whom this book is best suited. It has many strong chapters, especially on psychophysical topics, and these form a state-of-the-art coverage of an important part of the field. In other areas, things are more scattered, so that the book as a whole cannot be endorsed for an audience seeking a full general treatment. But that would be a great deal to ask of any volume of this sort. Perhaps the ideal reader of the book has the intellectual breadth and curiosity of Barlow himself, and the same inclination to absorb concepts and ideas from a loosely-knit but often fascinating collection of disciplines. □

J. Anthony Movshon is at the Center for Neural Science, New York University, 6 Washington Place, 8th Floor, New York, New York 10003, USA.

Pulitzer Prize

This year's Pulitzer Prize for general nonfiction has been won by zoologists Bert Hölldobler of the University of Würzburg and Edward O. Wilson of Harvard University for their monumental work *The Ants*.

The book, which was published in 1990 by Harvard University Press and Springer, runs to an astonishing 745 pages, has over 1,000 figures and includes all aspects of ant biology, evolution and ecology, with an emphasis on the reasons underlying the success and importance of the group. According to Donald Feener, Diane Davidson and Jon Seger who reviewed the book in these pages (see *Nature* **344**, 894–895; 1990), "Only Hölldobler and Wilson could have written such a comprehensive and integrated treatment of ant biology. It represents a herculean labour of love, and it sets a new standard for synthetic works on major taxa."

The bare bones

S. Mann

Skeletal Biomineralizaton: Patterns, Processes and Evolutionary Trends. Volumes I and II. Edited by J. G. Carter. *Van Nostrand Reinhold: 1991. Pp.1032. $99.95, £79.*

If you were choosing a book to slip into your briefcase intending to pull it out again during a long international flight to an annual conference, it would not be *Skeletal Biomineralization, Processes and Evolutionary Trends* edited by Joseph Carter. For a start, at over 1,000 pages, the book would make a large hole in your baggage allowance. More importantly, reading a book like this is a serious matter.

The book is big in every way, and I have to say that I find the editor's dedication to producing this tome as awe-inspiring as the subject matter itself. The book comes in two volumes. The first 600 pages of volume I contains 25 invited chapters overviewing the mineralogy and microstructure of calcified skeletal tissues. The scope of these chapters vary enormously; the first nine contributions are relatively short (8–15 pages) and cover various aspects of biomineralization including general mechanisms, biomechanics, and calcification in spicules, corals, sponges, arthropods and gastropods, for example. Chapters 10–16 are written by the editor and are concerned with shell microstructure — over 270 pages of immense detail. Thereafter, several shorter overviews, on subjects such as protochordate biomineralization, vertebrate skeletal tissues, dental structures and geology and related applications of isotopes, trace elements and amino acids, widen the perspective.

But all is not finished; another 200 pages including a glossary and a bibliography come before the index. The bibliography must rank as the best ever collated in this field. It is over 100 pages long and includes its own index. Volume II is more modest in size and provides supplementary illustrations, mainly in the form of photographs, of the skeletal structures discussed in volume I. The quality of production of electron micrographs, like that in volume I, is first class.

I was very impressed by this book. The work is scholarly and it is a mark of real scientific endeavour. Although the subject matter is very specialized, being at the more geological end of biomineralization, I recommend that chemists, materials scientists and biologists look at this book. The electron micrographs of volume II testify to the sophistication of biological processes to synthesize and assemble inorganic materials that would be termed smart in other more fashionable disciplines. This is a vast unexplored area and this book will be a valuable contribution not only in the detailed classification of skeletal structures but as a source of wonder and inspiration to scientists interested in the possibilities of mimicking biological materials in the laboratory. □

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Flights of the season

Jeremy J. D. Greenwood

Bird Migration. By Thomas Alerstam. *Cambridge University Press: 1991. Pp.420. £55, $105.*

MORE than anything else, the ability to fly characterizes birds. It imposes on them severe anatomical, physiological and ecological constraints. But it also gives them freedom — not just in a physical sense but ecologically, for they are able to exploit dispersed, patchy and variable resources in a way quite impossible for us earthbound mammals. Starlings clear one bird table and move on to the next; in the countryside they move from field to field, sometimes completely changing the direction in which they fly out from their roosts on successive days, as birds that encountered diminishing food supplies the day before follow their more successful fellows. In winters when their food supplies fail, the seed-eating birds of the northern forests, redpolls, crossbills, nutcrackers and waxwings, move far outside their normal ranges in search of food. Ptarmigan, breeding high in the mountains in summer, move down the slopes as winter advances. In Africa, queleas follow behind the moving rainbelts, so that they can feed on the flush of seeds produced in the few months after rain has fallen.

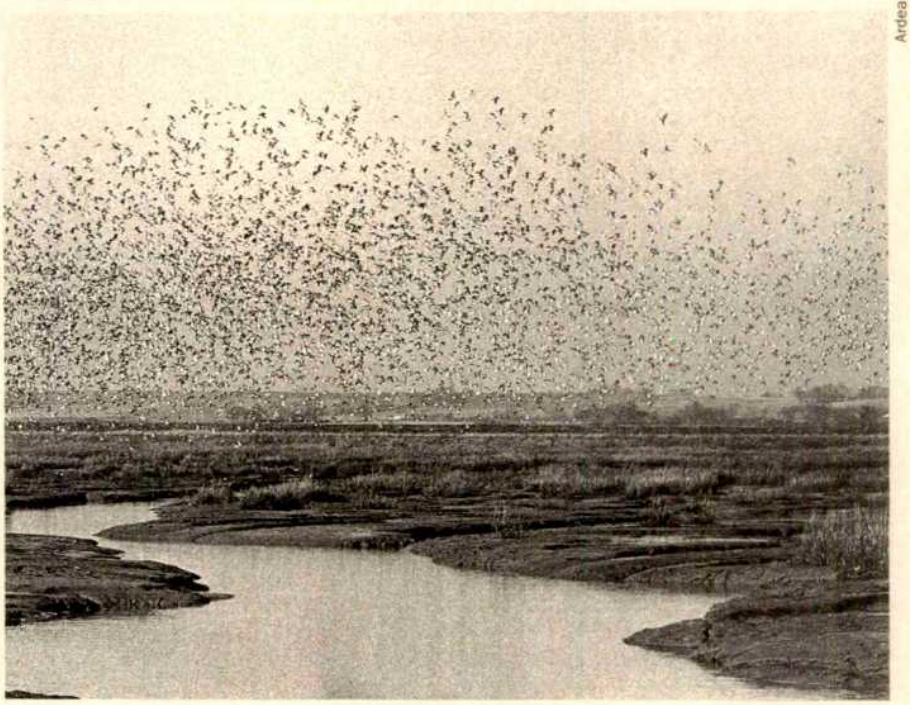
Virtually all bird migrations are seasonal. As Thomas Alerstam puts it in *Bird Migration*, "Birds migrate because we are tilted at 23.5°." He explains lucidly how this tilt and other aspects of the Earth's yearly round produce both the seasonal and geographical changes in temperature and rainfall that are at the root of migrations and the great wind belts that are so important in determining how particular journeys are made.

The journeys are often stupendous. Arctic warblers from southeast Asia, weighing under 10 grams, move 9,000 kilometres north to breed in the short summer of northern Scandinavia, much of their flight over the scarcely welcoming terrain of central Asia. Each autumn, thousands of millions of birds flood into subsaharan Africa from Europe and Asia. Typically, those from Europe cross the Mediterranean, North Africa and the Sahara in one hop of 50–70 flying hours. North Africa is too dry in autumn for it to be worthwhile stopping and the Saharan oases too few and too tiny. There is even evidence that sedge warblers fly directly to West Africa from southern England, a 70–90-hour journey at 50–60 kilometres per hour. They are generally boosted by favourable tail-winds but even so need to lay down so much fat and extra muscle that their weight at the start of the journey may be almost double its normal value. The winds are largely unchanged, and therefore adverse, in the spring but North Africa is now verdant, allowing the journey to be made in two stages. Similar journeys, but entirely over water, occur in the Americas. Columbus encountered large flocks of land birds midway between Bermuda and Puerto Rico, part of the great migration across the west Atlantic, which includes some small birds flying nonstop the 4,300 kilometres from Nova Scotia to Venezuela.

The exodus of many species, especially insectivores, from arctic, boreal and even temperate regions at the end of the summer is easily — perhaps too easily — explicable in terms of deteriorating food supplies and physical conditions. But why should they ever move north in the first place? If Africa can support them for part of the year, why not for the whole year? Does the answer simply lie in the otherwise unexploited abundant insect populations of the northern summer? Or is competition with African residents important? (And is competition with the migrants a reason for the low densities of some African residents?) Alerstam touches on these ecological questions largely on a case-by-case basis, in a long section in which he considers the migrations of individual species, grouped according to habitat and food. The details and diversity are fascinating, the anecdotes inspiring. Perhaps the diversity is too great for many broad principles to be extracted but one is left longing for a more synthetic treatment of the ecological factors that shape migration. Alerstam, not unreasonably, concentrates on the birds breeding in northwest Europe that he knows so well but one wonders if more ecological insights might emerge if one was to think of them not as northern birds that winter in the south but as southern species that spend a brief period of the year breeding in the north.

Turning to the journeys themselves, Alerstam gives a clear exposition of the ways in which birds fly and of how these influence migration strategies. The absence of thermals over water presents us with the great migration spectacles of thousands of soaring birds concentrating at narrow sea crossings at Gibraltar, the Bosphorus and elsewhere. He explains why birds use fat as fuel and how their varying abilities to store and use it determine the length and form of their journeys. He gives a detailed and fascinating account of how birds cope with vagaries of wind and weather, not just choosing under what conditions to migrate but responding during their flight to changing conditions and adaptively varying the altitude at which they fly. But even birds are not perfect and many are driven off course by adverse winds, to delight rarity-hunting birdwatchers.

There are other hazards too. Migrants crossing the Mediterranean have to run the gauntlet of Eleonora's Falcons, their own breeding delayed so that they can exploit the full potential of the huge autumn food supply. And some just get lost. Alerstam gives a fairly brief (though comprehensive and balanced) account of how birds orientate and navigate. The relatively low emphasis on this aspect is fortunate, for this is a fast-moving area of research and the book has been translated with minimal updating from the Swedish original, published in 1982. That it is somewhat out of date is a disadvantage but it remains useful. It presents both the basic principles and the fascinating natural history — and it does so in a style that preserves the wonder and magic of its subject. □



Ardea

Come fly away — flocks of waders setting out from Kent.

Jeremy J. D. Greenwood is at the British Trust for Ornithology, The Nunnery, Nunnery Place, Thetford Norfolk, IP24 2PU, UK.

The Earth's changing face

Mike Kirkby

World Geomorphology. By E. M. Bridges. *Cambridge University Press: 1990. Pp.260. Hbk £35, $70; pbk £13.95, $24.95.*

Temperate Palaeohydrology: Fluvial Processes in the Temperate Zone during the last 15,000 Years. Edited by L. Starkel, K. J. Gregory and J. B. Thornes. *Wiley: 1991. Pp. 548. £70, $175.*

As E. M. Bridges says in his introduction to *World Geomorphology*, there have been sweeping changes in understanding the Earth's crust since 1960 and some reluctance on the part of geomorphologists to digest the implications for the Earth's surface skin. Here was a perfect opportunity to provide new interpretations of major landform units, taking into account current views of plate tectonics, the great activity of the crust and our rapidly changing Pleistocene climates.

At continental scales, large-scale plate movements associated with sea-floor spreading provide passively extending continental margins like those around the Atlantic, and actively compressing margins as around the Pacific. Along passive margins, erosion and deposition lead to continental unloading and submarine loading, with consequent isostatic uplift and subsidence constrained by flexing of the elastic crust. These processes lead to the characteristic uplift of landward-dipping escarpments, increasing seaward dips in the rocks of continental coastal plains, and to the containment of catchments like the Mississippi or the Amazon which drain a large part of continental interiors. If thermal conditions in the crust are suitable it may eventually lead to progressive growth of the continent with the start of an active subduction zone.

At active margins, volcanic activity and compressive folds built mountain belts which are eroded to provide sediment which may be repeatedly recycled as it is returned to the continental margin on the subducting ocean plate. As the mountains are uplifted, their rocks are weakened by severe fracturing and the high elevations are also responsible for exceptionally high erosion rates, due to greatly increased precipitation and perhaps glaciation. Eventually the mountains become so steep and high that erosion rates balance uplift rates, perhaps even at the 20 mm per year reported for the New Zealand Alps. At smaller scales, the key interactions are between local tectonic stress fields, fault patterns and erosion, and there is much to be done before a clear understanding emerges, although some patterns may be seen, for example in block-faulted or rifted areas.

How far has Bridges achieved a worthwhile integration at the broad-scale implied by his title? Instead of a dynamic account, perhaps concentrating on those areas where most is known, we have an introductory chapter on plate tectonics at a broadly descriptive level, followed by a chapter on each of the six continents and on the Pacific Basin. For each continent, there is a token introduction on plate tectonics but rarely any dynamic view of the structure and form of the land. In fact there is very little fresh analysis and much that could have been written 50 years ago. The basic regionalization of the United States, for example, where much is now known, is still based on N. M. Fenneman's analysis of the 1930s and there is nothing on the dynamic behaviour of the mountain masses of the California coast.

Of course there are difficulties in attempting a region-by-region account of landforms across the world, but the resulting book frequently declines to the level of a catalogue of areas, with no space to give more than a bare description of each, and certainly no chance to present or discuss new insights. I hope that another author will take up the challenge.

Temperate Palaeohydrology edited by L. Starkel, K. J. Gregory and J. B. Thornes might appear to have a similar sweep in scope, with its 15,000-year time span, but its aims are more modest in scope. It is the concluding volume in a series of edited contributions arising from a project undertaken by the Internation Geological Correlation Programme over the last decade. The theme is introduced by chapters on river hydrology and the sedimentology of river deposits. Over half of the book consists of a series or regionally based case studies which span Europe. There are three studies on Britain, two each on Finland, Benelux, Poland and the Danube basin, and one each on the eastern Baltic, Switzerland and western Siberia. In all cases the crucial evidence relates to dating and correlation of buried channels and terraces with other forms of evidence for changes in relative sea levels and erosion rates. Several of the studies are for areas of glacial isostatic rebound. Another group is for areas influenced by glacial meltwater or ice damming, and there is a group of rivers which were not directly influenced by glaciation.

The final 150 pages of the book provides some broader perspectives. The most substantial is a survey of methods for retrodicting former discharges from the sedimentological record, with the implications for the associated hydrological balances for the immediately postglacial period, when discharges were several times greater than today. The changes are attributed mainly to the lower evapotranspiration in cooler conditions rather than to major changes in precipitation. There are also useful perspectives on glacier fluctuations, and on the possibilities for broad correlations across the whole area of Europe, among others. Like many other contributed collections, *Temperate Palaeohydrology* gives a fair view of the state of the art in a particular field, but emphasizes the diversity of activity rather than providing broad insights which could be carried back to the global scale attempted in *World Geomorphology*. □

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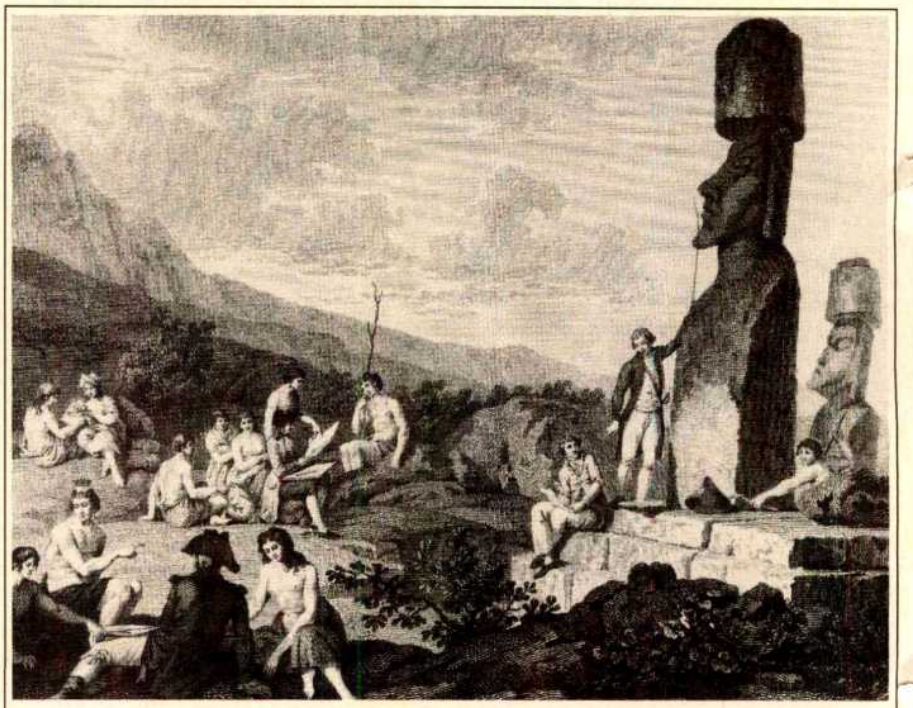

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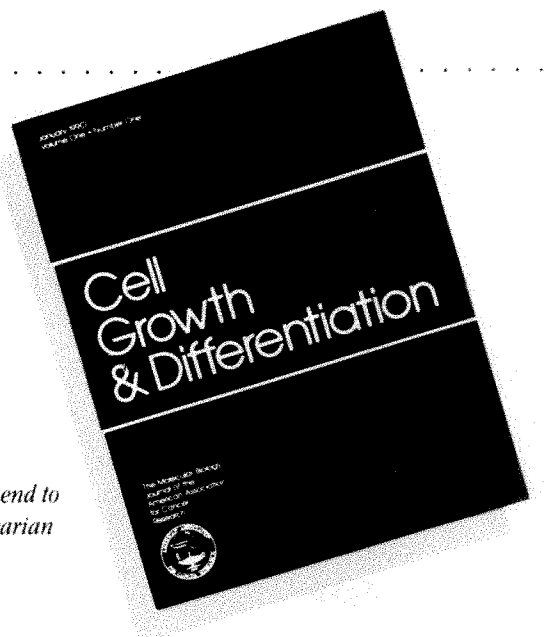

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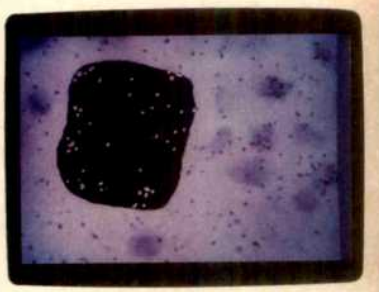

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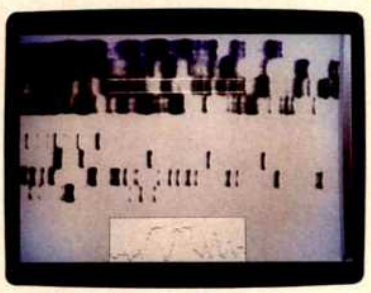

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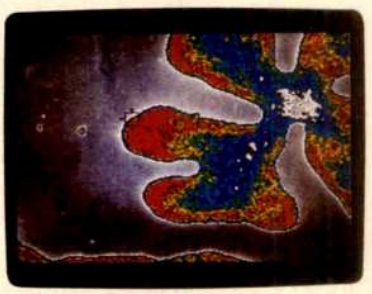

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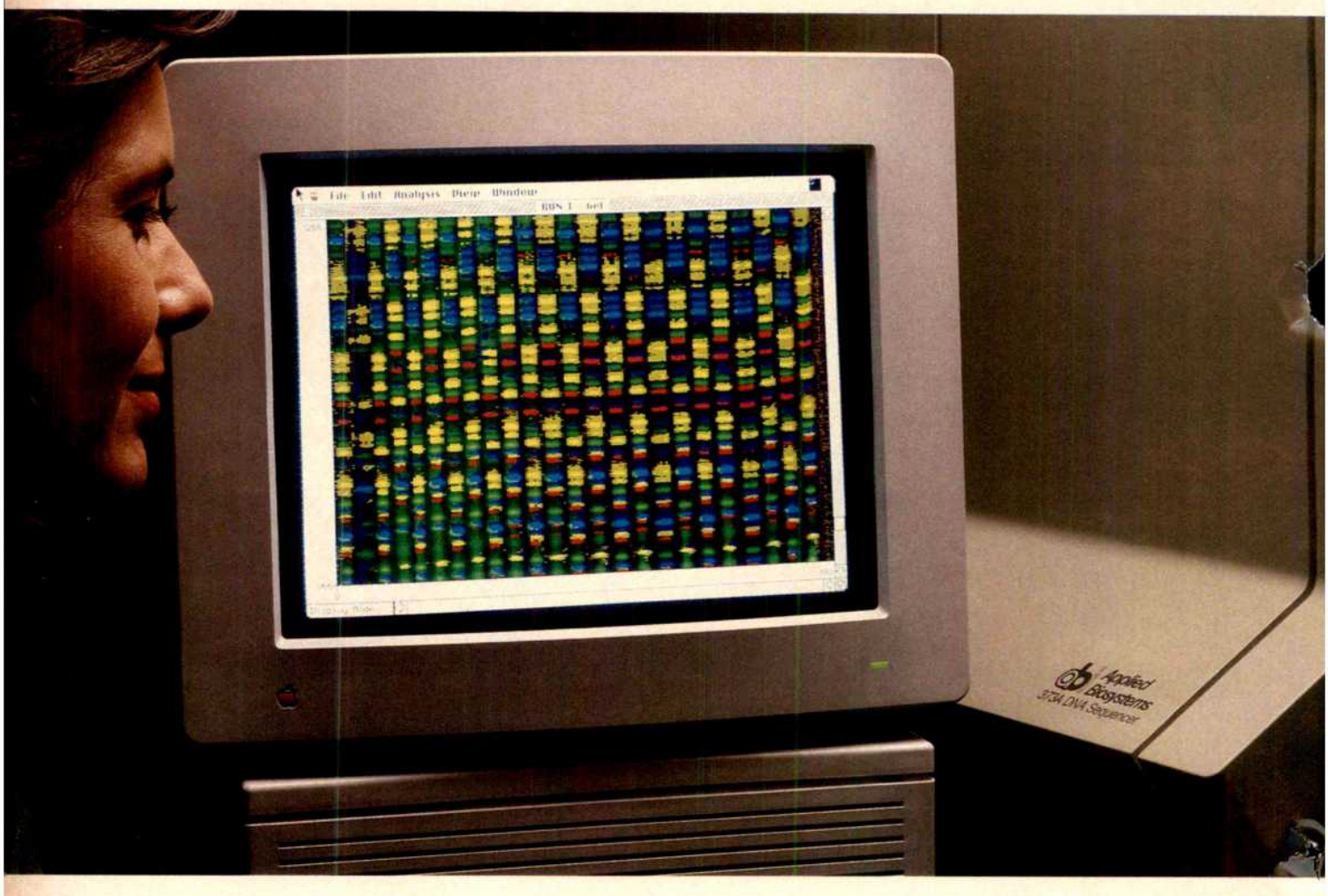

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Thermodynamic regulation of ocean warming by cirrus clouds deduced from observations of the 1987 El Niño

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Observations made during the 1987 El Niño show that in the upper range of sea surface temperatures, the greenhouse effect increases with surface temperature at a rate which exceeds the rate at which radiation is being emitted from the surface. In response to this 'super greenhouse effect', highly reflective cirrus clouds are produced which act like a thermostat, shielding the ocean from solar radiation. The regulatory effect of these cirrus clouds may limit sea surface temperatures to less than 305 K.

WATER vapour and clouds are the dominant regulators of the radiative heating of the planet. General circulation model studies have identified the complex effects of the various cloud and water vapour feedbacks and their importance to climate change[1-3]. We do not understand how these radiative effects respond to a climate change, nor do we understand their feedback effects. What we need is a natural climate-change experiment in a region that exhibits a strong coupling between surface temperatures and radiative fluxes. The tropical Pacific shows such behaviour. In particular, during the El Niño events which occur once every 2–6 years[4], the equatorial Pacific warms by as much as 2–4 K, and the warming is accompanied by marked variations in the radiation fluxes at the top of the atmosphere[5]. Accurate data on the radiation budget from the Earth radiation budget experiment (ERBE)[6] and sea-surface-temperature (SST) data from weather satellites and ships[7] are available for the 1987 El Niño[8]. We use this data set to explore the link between ocean warming and the radiative feedbacks. Relevant definitions are given next.

The total greenhouse effect of the atmosphere and clouds, G, is defined[9] as $G = E - F$, where F is the radiation emitted to space, and $E = \sigma T^4$ is the energy emitted by the ocean surface, with $\sigma = 5.67 \times 10^{-8}$ W m^{-2} K^{-4} and T = SST. Observations of F over the clear-sky regions, F_c, are employed to obtain the atmospheric portion of the greenhouse effect, $G_a = E - F_c$. The difference between G and G_a gives the enhancement of the greenhouse effect caused by clouds, or 'cloud long-wave forcing', $C_l = G - G_a = F_c - F$. Clouds also enhance the albedo (reflectivity) of the planet, thereby decreasing the planetary solar heating. This effect, the 'cloud short-wave forcing' (ref. 6), C_s, is estimated by subtracting the solar energy absorbed by a region from that over just the clear-sky portion of that region. Hence, the absorbed solar energy can be expanded as $S(1-A) = S_c + C_s$, where S is the solar radiation at the top of the atmosphere, A is the column albedo, and S_c is the clear-sky solar absorption. ERBE measurements yield the fluxes for the top of the atmosphere of F, F_c, S and S_c together with the albedo A. Thus we have observable definitions for the various feedback terms.

During the El Niño of 1987, the equatorial Pacific warmed by as much as 3 K (Fig. 1*a*). The warming was accompanied by a significant increase in the greenhouse effect of the atmosphere (Fig. 1*b*) and that of the clouds (Fig. 1*d*). These positive feedback effects are offset by a significant decrease in the absorbed solar energy (Fig. 1*c*). We show that this cirrus regulation of ocean warming is triggered primarily when SSTs exceed 300 K. Results of past studies[9-13] enable us to conclude that the interactions between SST, deep convection, large-scale transport of moisture and cirrus formation are responsible for the observed negative feedback effect of cirrus.

Analyses of the interactions

We begin with the net radiative heating of the surface–atmosphere system, $H = S(1-A) - F$. Adopting the previous definitions for $F = \sigma T^4 - G_a - C_l$ and $S(1-A) = S_c + C_s$, we have

$$H = S_c - \sigma T^4 + G_a + C_l + C_s \qquad (1)$$

The absorbed solar radiation, S_c, heats the surface, which emits the energy as long-wave radiation, σT^4. An amount G_a is

TABLE 1 Summary of feedback terms

a, Experiment 1: spatial and seasonal

Domain	Period (month/year)	dG_a/dT (W m^{-2} K^{-1})	dC_s/dC_l
25° N–25° S	4/85	5.6†	−1.07*
	4/87	6.5†	−1.03†
	2/87	6.9†	−0.96*
	2/88	6.3†	−0.94*
10° N–10° S	4/85	7.2†	−1.13*
	4/87	8.1*	−1.12*
	2/87	8.2*	−0.9†
	2/88	8.5†	−0.9†
0–25° N	2/85	4.6*	−1.01*
	4/85	5.2†	−1.26*
0–25° S	2/85	7.4†	−1.03†
	4/85	6.5†	−0.87*
10° N–10° S	4/85; 2/87; 2/88; 4/89	7.7*	−0.95†
Atlantic	Same	7.9*	−1.03†
Indian Ocean	Same	8.1‡	−1.03†

b, Experiment 2: El Niño

Domain	Period (month/year)	dG_a/dT (W m^{-2} K^{-1})	dC_s/dC_l	dC_s/dG	a_s (W m^{-2} K^{-1})	a_l
10° N–10° S	{4/87–4/85; 4/89–4/87; 2/88–2/87}	6.8*	−1.2*	−0.92†	−22‡	18*
	4/87–4/85	9.2*	−1.33†	−0.97*	−20*	16*
	4/89–4/87	8.5*	−1.23†	−0.95†	−19‡	17*
	2/87–2/85	6.4‡	−1.09†	−0.97†		
	2/88–2/87		−1.11*	−0.97*		
	5/87–5/85	8.0*	−1.22†	−0.92†		
	7/87–7/85	5.7*	−1.17†	−0.79‡		
	10/87–10/85	6.0*	−1.32†	−0.99*		

Unless otherwise stated, the domain is the Pacific Ocean stretching from 140° E (western Pacific) to 90° W (eastern Pacific). SST is denoted by T; the atmospheric greenhouse effect by G_a; the cloud long-wave forcing is C_l; the total greenhouse effect is $G = G_a + C_l$; the cloud modulation of absorbed solar energy is the cloud short-wave forcing C_s; and the cloud-forcing slopes a_s (short-wave) and a_l (long-wave) are obtained from Fig. 4*a* and *b*, respectively.

* Correlation coefficient $|r|$ for least-squares linear fit is in range $0.9 > |r| > 0.8$

† $|r| > 0.9$

‡ $0.8 > |r| > 0.7$. When $|r| < 0.7$, the correlation is considered insignificant and is omitted from the table.

trapped in the atmosphere, thus reducing the cooling to space. The long-wave energy flow out of the system is further reduced by the flux C_l trapped in clouds. But this heating effect is offset by the reduction C_s of solar absorption by clouds with $C_s < 0$. We wish to examine how G_a, C_l and C_s vary with T. These dependences are obtained both from the spatial and seasonal variations in T and from the 1987 El Niño, which began during late 1986.

We use ERBE measurements for 1985–89. The SSTs are obtained from combined analyses from the National Oceanic and Atmospheric Administration and the National Meteorological Center[7] of satellite and *in situ* data. The data are monthly averaged values with a spatial resolution of 2.5° (latitude) × 2.5° (longitude). The analyses will focus primarily on April, but we will briefly review results for the months of February, May, July and October. April is preferred for several reasons. Because of the near-hemispherical symmetry of solar radiation and of meteorological features in the equatorial regions, we can safely ignore hemispherical asymmetries in the dependence on the solar zenith angle before comparing the albedos of the different oceans. Around April, equatorial SSTs reach peak seasonal values and exceed 300 K over most of the Pacific[4], the minimum SST necessary to initiate deep convection. Finally, the 1987 El Niño warming stabilized at near-maximum values around April 1987.

Experiment 1

In this experiment, we examined spatial variations in the greenhouse effect and cloud forcing for different seasons. The total greenhouse effect $G = G_a + C_l$ increases sharply when the SST exceeds ~300 K (Fig. 2*a*), a feature that is present in all the months and can also be inferred from earlier studies[14,15]. Optically thick cirrus clouds in the upper troposphere are needed to account for the large increase in G of ~100 W m^{-2} as the temperature increases from 300 to 303 K. The slope, dG_a/dT, for the tropical Pacific (10° N to 10° S) is ~6–9 W m^{-2} K^{-1} (Fig. 2*b*, and Table 1), whereas it is ~3.4 W m^{-2} K^{-1} for the entire Pacific (60° N to 60° S). About half of the 3.4 W m^{-2} K^{-1} is due to atmospheric trapping of the increased black-body emission. The balance is due to water vapour feedback[9]: the warmer atmosphere contains more water vapour which in turn traps more radiation. The fact that $dG_a/dT > 6$ W m^{-2} K^{-1} has important climate implications.

The surface emission increases at a rate close to $dE/dT = 4\sigma T^3 \approx 6.1$ W m^{-2} K^{-1} at $\sim T = 300$ K. But as $dG_a/dT \geq dE/dT$, the energy cannot escape to space but instead is trapped in the atmosphere. Indeed, the slightly larger slope of G_a suggests that the trapping increases faster than the surface emission. The main deduction is that the warmer tropical-ocean/atmosphere system has lost the fundamental negative feedback between temperature and infrared emission which expels excess heat by radiating to space. This tropical 'super greenhouse effect' is caused by a combination of several mutually reinforcing factors, including increases in total-column H_2O[9,10,16]; H_2O continuum absorption[17] which scales quadratically with H_2O partial pressure; higher middle- and upper-troposphere H_2O concentrations[19], and changes in the lapse rate[18,19]. The system is potentially unstable unless another negative feedback exists to stabilize it,

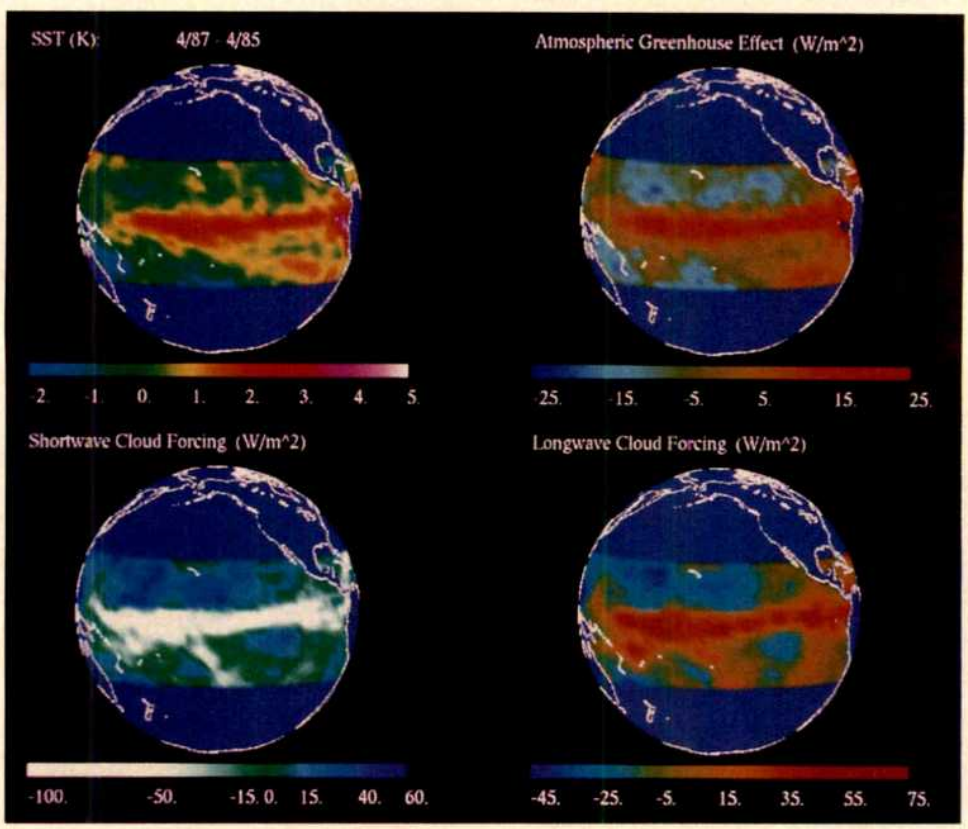

FIG. 1 Changes in the SST, the greenhouse effect and the cloud forcing accompanying the 1987 El Niño. Top left panel (*a*), April 1987 minus April 1985 monthly mean SSTs for 25° N to 25° S. Outside this region changes are much smaller. SST data are obtained from ref. 7. The equatorial warming began during late 1986 and was well established by April of 1987 (ref. 8). Top right panel (*b*), April 1987 minus April 1985 atmospheric greenhouse effect. Bottom left panel (*c*), April 1987 minus April 1985 cloud short-wave forcing. The El Niño warming leads to highly reflective clouds which reduce (as $C_s < 0$) the solar energy absorbed by the oceans by as much as 100 W m^{-2}. Bottom right panel (*d*), April 1987 minus April 1985 cloud long-wave forcing, C_l. The greenhouse effect of clouds increases significantly over the regions subject to the surface warming. Increased cirrus cloudiness in the upper troposphere is needed to account for changes in C_l larger than 30 W m^{-2}.

and this brings us to the changes in the cloud forcing. The cloud-forcing terms C_l (Fig. 1*d*) and C_s (Fig. 1*c*) also increase in magnitude for warmer oceans (Fig. 1*a*). A closer examination of the relationship between C_l and C_s (Fig. 2*c*), reveals the strong degree of anti-correlation between the two, which can also be deduced from results published earlier[20-22]. The generality of this finding is shown in Table 1. This remarkable correlation and the fact that large negative values of C_s are correlated with warmer waters (Fig. 1*d*) lead to the following conjecture: as the tropical oceans warm, the rapid rise of G_a with SST leads to an unstable feedback. The warming continues until the clouds become thick enough to shield the ocean from the solar radiation and arrest further warming. This thermostat effect of the cirrus anvils controls the maximum value of SSTs. The validity of this inference is examined next.

Experiment 2

In this experiment, we examined the flux changes that occurred during the 1987 El Niño. The observed changes are very similar to those inferred above. First, G_a increases with SST (Fig. 3*a*) at a rate of 6–9 W m^{-2} K^{-1}. The plot of dC_s against dC_l (Fig. 3*b*) confirms that clouds with large C_l are also very reflective. As $dC_s/dG \geq -1$, the decrease in the absorbed solar energy nearly balances the increase in the total greenhouse effect (Fig. 3*c*). These results support the inference from experiment 1 that G increases significantly with a warming of the ocean, and this unstable increase is effectively offset by the brighter clouds resulting from the warming. Similar results are obtained for the months of February, May, July and October (Table 1), but results for February and July should be viewed with caution as we have not accounted for seasonal changes in the incoming solar radiation and the solar zenith angle, both of which have complex and largely unknown effects on the cloud-forcing terms.

To probe further into the link between the warming and the absorbed solar energy, we plot the product of dT and dC_s against $(dT)^2$ for each 2.5° × 2.5° region (Fig. 4). If the relationship is linear, we can obtain the rate of decrease of C_s with increase in SST from the slope, a_s. A statistically significant relationship does exist for April (Fig. 4 and the column under a_s in Table 1), although it is weaker for the other months; for example, for February 1987 minus February 1985 and for May 1987 minus May 1985, a_s is ~ -15 W m^{-2} K^{-1}. The correlation coefficient $|r|$ is only ~0.6 and is almost zero for July and October. But we may obtain a better picture by examining the relationship on a larger scale.

We focus on the region of maximum warming, the central and eastern Pacific from 180° (the date line) to 90° W and 5° N to 5° S. During April, for example, the average SST for this region increased from 300.1 in 1985 to 301.9 K in 1987 and the average C_s changed from −29 in 1985 to −63 W m^{-2} in 1987 ($dC_s = -34$ W m^{-2}); for February, SST increases from 299.2 to 301 K and $dC_s = -32$ W m^{-2}; for May, SST increases from 300 to 301.7 K and $dC_s = -34$ W m^{-2}; for July, the changes are from 299 to 301 K and $dC_s = -12$ W m^{-2}; and finally for October, SST increases from 298.6 to 300.3 K and $dC_s = -6$ W m^{-2}. Several inferences can be made. First, on the larger scale the warming is accompanied by a decrease in solar absorption without exception. This is reassuring as the processes that link SST with C_s involve cooperative interaction between deep convection and the large-scale circulation which transports moisture into the region of warming from the rest of the tropical Pacific (details given later). Second, if we exclude the colder eastern Pacific from 90° W to 120° W, which is mostly devoid of deep convection, dC_s for October decreases from −6 to −13 W m^{-2} with similar decreases (ranging from −5 to −9 W m^{-2}) during other months. This is another indication of the role of deep convection in triggering the negative feedback between SST and C_s. Finally, the marked decrease of $-dC_s$ towards October, which roughly marks the final phase of the El Niño, is a reminder of the transient nature of the event. As the El Niño warming is

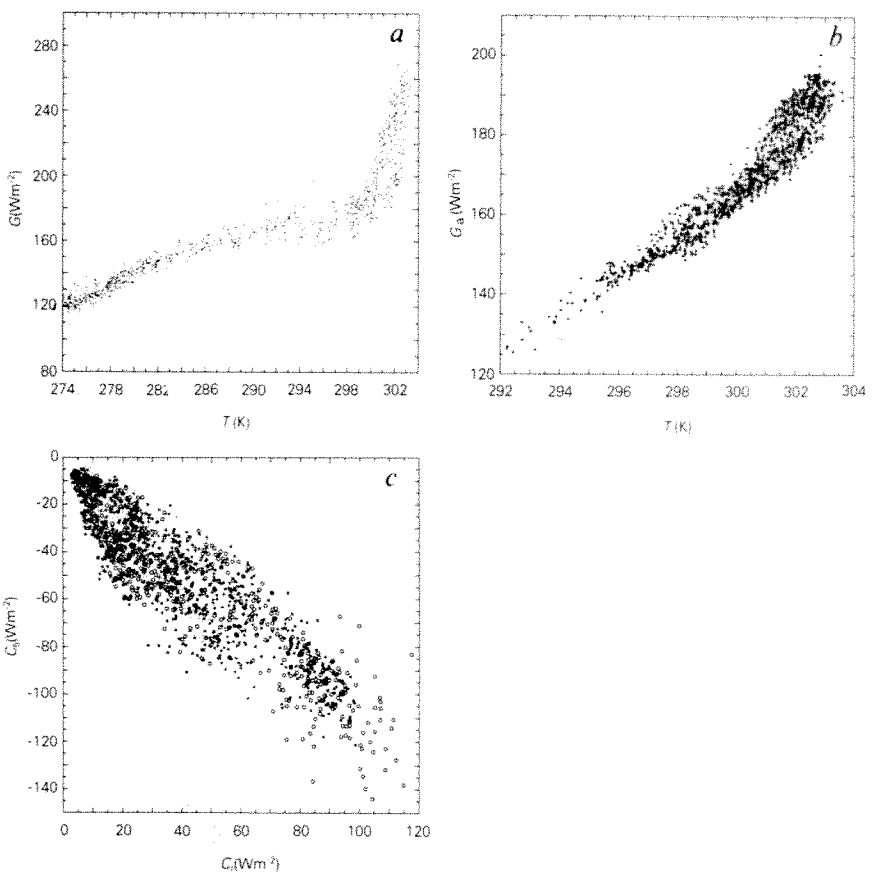

FIG. 2 Monthly mean greenhouse effect and cloud-forcing terms for the Pacific extending from 140° E to 90° W. Each point in this and other figures represents a monthly averaged value for a 2.5° (latitude) × 2.5° (longitude) region. *a*, Total greenhouse effect, G, plotted as a function of SST for April 1987 from 85° N to 85° S. Regions with SST < 274 K are excluded. *b*, Atmospheric greenhouse effect G_a against SST for the tropical Pacific from 25° N to 25° S for April 1987. The least-squares linear slope is $dG_a/dT = 6.5$ W m^{-2} K^{-1} with a correlation coefficient of $r = 0.96$. *c*, Cloud short-wave forcing against cloud long-wave forcing for the region 10° N to 10° S for 4/85(+), 2/87(○), 2/88(×), and 4/87(·). The linear correlation is $dC_s/dC_l = -0.951$ ($r = -0.9$).

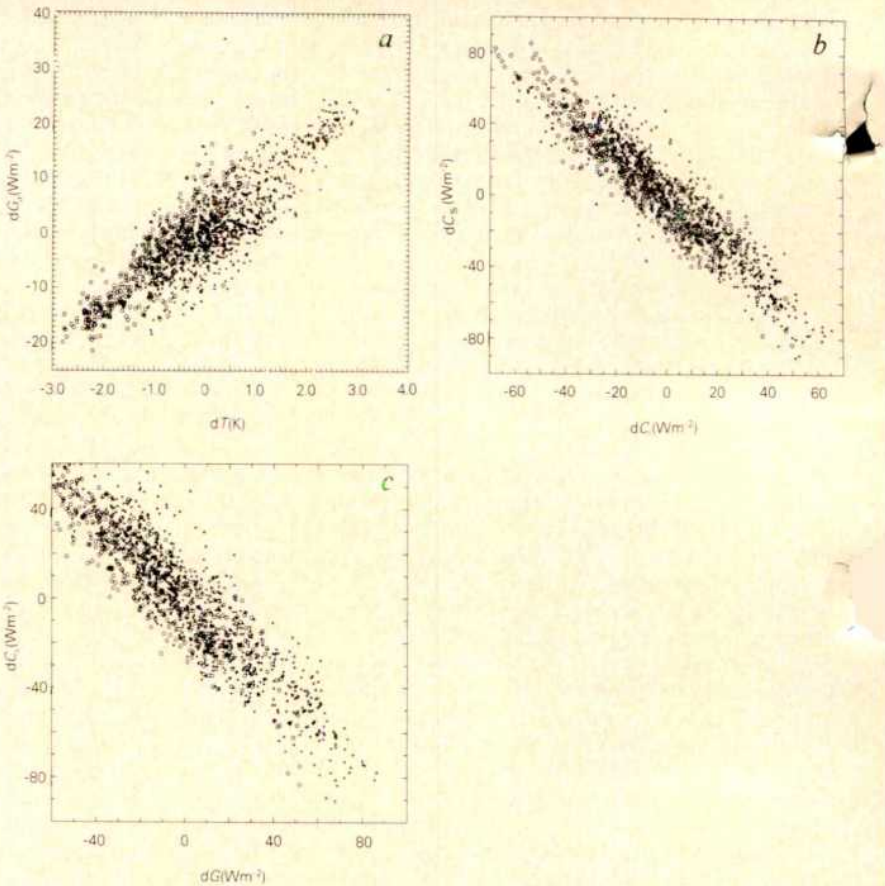

FIG. 3 Changes induced by 1987 El Niño in monthly mean greenhouse effect, cloud-forcing terms and SST for the Pacific ocean (140° E to 90° W) from 10° N to 10° S. The points represent the differences for 4/87 minus 4/85(+), 4/89 minus 4/87(○), and 2/88 minus 2/87(×). *a*, dG_a against dT where d denotes difference between two years. The linear fit is $dG_a/dT = 6.80\ W\ m^{-2}\ K^{-1}$ ($r = 0.85$). *b*, Change in cloud short-wave forcing against change in cloud long-wave forcing. The linear fit is $dC_s/dC_l = -1.20$ ($r = -0.93$). As $dC_s/dC_l < -1$, clouds have a net cooling effect. *c*, Change in cloud short-wave forcing against change in total greenhouse effect $G = G_a + C_l$. The linear fit is $dC_s/dG = -0.92$ ($r = -0.92$), which indicates that the increase in the greenhouse effect is slightly larger than the decrease in solar absorption.

a transient phenomenon which started to revert back to 'normal' conditions around November 1987, considerable changes in the equatorial dynamics during the terminal phase of the El Niño[13] can be expected to modify the radiative feedbacks.

To explore additional climate implications, we estimate the change of C_s with SST, although acknowledging that this is a hazardous task given the nonlinear nature of the coupling between SST, ocean–atmosphere dynamics and radiation physics. We can use two independent methods: (1) From Fig. 3*a*, $dG_a/dT \approx 6.8\ W\ m^{-2}\ K^{-1}$. As $dC_s = -0.92\ (dC_l + dG_a)$ (Fig. 3*c*) with the further constraint that $dC_s = -1.20\ dC_l$ (Fig. 3*b*), we obtain $dC_s/dT \approx -27\ W\ m^{-2}\ K^{-1}$ and $dC_l/dT \approx 23\ W\ m^{-2}\ K^{-1}$. (2) The covariance plot (Fig. 4*a*) of dC_s and dT with $(dT)^2$ and the corresponding plot for dC_l (Fig. 4*b*) yield $dC_s/dT \approx -22$ and $dC_l/dT \approx 18$, where because of uncertainties in the SST data, regions with $dT < 1$ K are excluded.

Thermodynamics and dynamics of the feedbacks

In what follows, we describe the fundamental principles that govern the observed correlations described above. In the warm climate regimes of interest to this study, the thermodynamics of H_2O have a dominant influence on the radiative feedbacks. The saturation humidity, q_s, depends exponentially on temperature as $\{e^{(-5,400/T)}\}/T$ (T in kelvin). As a result, a 1% increase in T from $T = 300$ K, for example, increases q_s by 17%. Indeed, satellite observations[9,10] reveal that total atmospheric water vapour increases by ~17% per 1% increase in SST. Hence G_a increases with SST.

The latent energy of a parcel of air also grows by 17% per 1% increment in T. When $T > 300$ K, a parcel of moist air near the surface has sufficient latent energy that if it is forcibly lifted until it reaches saturation, it can overcome the gravitational potential energy and rise to the upper troposphere as a cumulonimbus cloud[11,12]. The ice crystals detrained by these deep clouds spread thousands of kilometres downwind in the form of cirrus anvils[22,23] and also moisten the upper troposphere[18]. The maxima in the cloud-forcing fields (Fig. 1) are caused by the cirrus anvils[12] and not by the cumulonimbus clouds, which are two orders of magnitude smaller[23]. When SST drops below 300 K, the latent energy in the surface layer is generally not sufficient to form deep convection[24]. Hence G increases rapidly when $T > 300$ K.

Consider next the total static energy of the atmosphere, $h = C_pT + gZ + Lq$, where the first term is the thermal energy, the second is the gravitational potential energy and the third is the latent energy, with Z the altitude and q the H_2O mass mixing ratio. We rewrite it in the form $h/C_p = T + (g/C_p)Z + (L/C_p)q$ where $g/C_p = 9.8\ K\ km^{-1}$ and $L/C_p = 2{,}500$ K. At $T = 303$ K, the saturation q at the surface is 0.026, and assuming a reasonable value of 75% for the relative humidity, the latent energy is ~50 K and $h/C_p = 353$ K. If we adopt the observed equatorial temperature and humidity profiles[25], h/C_p decreases from 353 K at $Z = 0$ km to 330 K at $Z = 6$ km. Above 6 km it increases with Z to ~350 K at 16 km because of the dominance of the potential energy term. As a result, a surface parcel with SST = 303 K can penetrate to ~16 km. When the SST drops to 297 K, the surface parcel has a static energy of only 330 K and can barely reach the middle troposphere.

The warmer ocean thus produces clouds at higher altitudes. As cloud-top temperature and emission of radiation to space decrease with altitude, these clouds trap more long-wave radiation and have a larger greenhouse effect[26]. As a result C_l can increase with SST. Why does the solar reflection increase with SST? Deeper penetration by clouds is not sufficient to account for the enhanced solar reflection; in addition, the extent of cloudiness and the optical thickness of the clouds have to increase with SST. The required moisture for sustaining cirrus is not necessarily provided by local evaporation[27] but instead by large-scale transport within the lower troposphere into the region of convection[27,28]. These large circulation systems are the

'Hadley' and 'Walker' circulations[4]. The sources of energy for these large-scale motions are the latent heat released by convection[29], the cirrus long-wave cloud forcing and the spatial gradients in SST[30]. Therefore this convective large-scale system is self-sustaining. The large-scale convergence of moisture into the warm oceanic regions amplifies the warming through the enhanced atmospheric greenhouse effect, further driving the circulation. This continues until the cirrus clouds, which accumulate during this process, reflect enough sunlight to arrest further warming. Thus the anvils act like a thermostat.

Predictions of the thermostat hypothesis

The independent natural experiments support this hypothesis: the feedback between SST, convection and cloud reflection of solar radiation prevents a runaway greenhouse effect on the SST and acts as a thermostat to regulate the maximum ocean temperatures on the planet. The climate implications of this hypothesis are now developed in more detail.

Maximum ocean temperature. Let us consider an ocean region where dynamical transport of heat in the surface layers is negligible so that it can reach the maximum value imposed by the solar absorption. The equatorial western Pacific nearly satisfies this criterion because the net heat flux into the ocean is very small (0–20 W m^{-2})[31,32]. We can therefore apply equation (1) with a correction for atmospheric transport which is discussed next. An examination of the equatorial temperature gradients[25] reveals that the mid-to-upper troposphere has negligible east-west and north-south gradients in spite of the fact that the latent heating (200–300 W m^{-2})[4,31] and cloud greenhouse effect add as much as 300–400 W m^{-2} heat flux in the western portions of the Pacific. Seasonal and longitudinal average temperatures in the upper troposphere differ by less than 2 K between 25° N to 25° S. Likewise, the upper troposphere in the equatorial western Pacific is less than 2 K warmer than that of the eastern Pacific 10^4 km away. This homogeneity follows from the balancing of latent and radiative heating in the tropical atmosphere by adiabatic cooling resulting from convective and large-scale overturning[33]. In particular, adding energy to the upper troposphere is very effective in driving the Walker and the Hadley cell circulation[29]. Recent general circulation model studies[34,35] also suggest that the radiative energy converging in the anvils does not cause localized warming over the anvil regions. Instead, the warming is uniformly distributed over the entire tropical region.

We therefore assume that the C_i term, which is largely heat trapped in the troposphere, is transported out of the western Pacific, and only a small fraction contributes to warming the ocean locally. This fraction, f, ranges between 0 and 0.2. The upper bound is related to the fraction of the long-wave emission from the base of cirrus clouds that can reach the ocean surface directly. Incidentally, C_i (~60–80 W m^{-2}) is nearly equal to the net annual mean radiative heating of 80 W m^{-2} (ref. 36) at the top of the atmosphere minus the oceanic heat transport[32] of ~0–20 W m^{-2} in the western Pacific region. This implies that 60 W m^{-2} is being transported out of the region by advection within the atmosphere, which is consistent with the assumed range for f.

On the other hand, the C_s term represents a direct loss of solar energy to the ocean surface[34]. With the above inferences, equation (1) can be rephrased as follows:

$$\mathrm{SST}_{max} = T_0 + \{(S_c + G_0 - \sigma T_0^4)/\beta\} \quad (2)$$

$$\text{where } \beta = 4\sigma T_0^3 - \mathrm{d}G_a/\mathrm{d}T - f\,\mathrm{d}C_i/\mathrm{d}T - \mathrm{d}C_s/\mathrm{d}T$$

The net radiative feedback is denoted by β. The variables have been expanded about a reference temperature T_0; for example, $G_a = G_0 + \mathrm{d}G_a/\mathrm{d}T$, where $G_0 = 165$ W m^{-2} is the value of G_a at T_0, assumed to be 300 K. It is safe to assume an uncertainty of ~±15 W m^{-2} in the radiative flux terms. The clear-sky solar absorption is $S_c = S(1 - A_c) = 370$ W m^{-2}, where $S = 424$ W m^{-2} is the annual mean equatorial solar radiation and $A_c = 0.1$ is the clear-sky albedo, and we subtract 12 W m^{-2} for stratospheric solar absorption[37]. Similarly, the stratospheric contribution of 5 W m^{-2} to G_a has been subtracted from G_0. The values derived from Figs 3 and 4 are adopted for the various feedback terms in β. Changes in the equatorial atmospheric temperature and humidity profiles in response to SST changes are implicitly included in the long-wave feedback terms, as $\mathrm{d}G_a$ and $\mathrm{d}C_i$ are obtained directly from observed changes. The cloud feedback terms arising from convection will be zero when $T < T_0$, and the black-body term is $4\sigma T_0^3 = 6.1$ W m^{-2} K^{-1}. Note first that in the absence of the cloud feedback terms, the black-body negative feedback (the first term in the denominator) is dominated by the positive feedback effect of $\mathrm{d}G_a/\mathrm{d}T$ (6.8 W m^{-2} K^{-1}), creating a potentially unstable state. Substitution of $-27 < \mathrm{d}C_s/\mathrm{d}T < -22$ and $18 < \mathrm{d}C_i/\mathrm{d}T < 23$ yields:

$$303 < \mathrm{SST}_{max} < 305 \text{ K}$$

The lower limit is for $f = 0.0$ and $\mathrm{d}C_s/\mathrm{d}T = -27$, and the upper limit of 305 K is for $f = 0.2$ and $\mathrm{d}C_s/\mathrm{d}T = 22$ W m^{-2} K^{-1} (from Fig. 3). If we allow 20 W m^{-2} for oceanic heat transport, the 305 K value drops to ~304 K and the 303 K value drops to 302 K in agreement with observed western Pacific SSTs (Fig. 2*a*). For the above example, the feedback value ranges between $15 < \beta < 26$ W m^{-2} K^{-1}. This large damping should tend to minimize the seasonal variations of the SST in the western Pacific.

Evaporative cooling of the warm oceans is another possible mechanism[15,25] for limiting SSTs, but this cannot be a limiting factor for several reasons: (1) the added moisture to the boundary layer will enhance G_a; and (2) observed precipitation exceeds evaporation by nearly a factor of 2 in the western Pacific[4,25,31] (the western Pacific ocean imports rather than

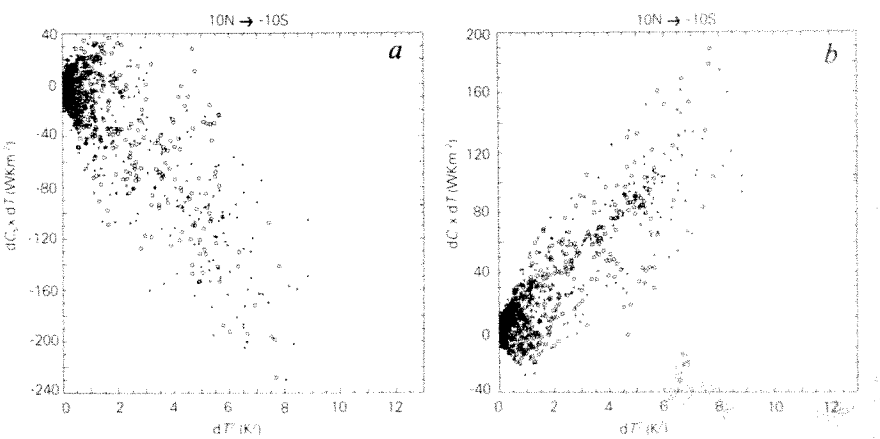

FIG. 4 *a*, Change induced by 1987 El Niño in solar absorption by the ocean–atmosphere system. Product of $\mathrm{d}C_s$ and $\mathrm{d}T$ against $(\mathrm{d}T)^2$ for the Pacific, 10° N to 10° S. The points represent the differences between 4/87 and 4/85(+) and 4/89 and 4/87(○). A negative value for the product indicates a decrease (increase) in absorbed solar energy with a warming (cooling) of the ocean. The linear fit is $\mathrm{d}C_s/\mathrm{d}T = -19$ W m^{-2} K^{-1} ($r = -0.80$). The error in SST data is 1 K, and when regions with $\mathrm{d}T < 1$ K are excluded the fit is $\mathrm{d}C_s/\mathrm{d}T = -22$ W m^{-2} K^{-1} ($r = -0.73$). *b*, Same as (*a*) but for cloud long-wave forcing. The linear fit is $\mathrm{d}C_i/\mathrm{d}T \approx 16$ and increases to 17.5 W m^{-2} K^{-1} when points with $\mathrm{d}T < 1$ K are excluded.

exports water vapour). This inference, albeit based on imprecise observations, is also substantiated by the low salinity of the surface water[31]. What is needed is a permanent loss of energy, and reflection of sunlight back to space is the most efficient mechanism to accomplish this task.

El Niño warming. The basic hypothesis should apply during an El Niño as well. Indeed, during both the 1987 El Niño and the more severe 1983 El Niño[4], the maximum SST did not exceed 303 K. In the central and eastern Pacific, upwelling of colder water from below and advection of cold water from the eastern coast causes SSTs to drop below 303 K (refs 4, 31). When the dynamical cooling effects abate during periods such as an El Niño year, the SST approaches the maximum value permitted by the anvil thermostat. Thus the El Niño year constitutes the equilibrium state of the Pacific, whereas the non El Niño years are the periods when the central and east Pacific is pulled away from equilibrium by the ocean dynamics.

Greenhouse and solar-dominated climate regimes

For the oceans[9], the globally averaged greenhouse effect (G) is 175 W m^{-2} and the absorbed solar radiation is 240 W m^{-2}. For the warmest equatorial oceans, the maximum values are $G \approx 280$ (Fig. 2*a*) and solar absorption ~300 W m^{-2} (ref. 36). In the absence of clouds, S can be as large as 380 W m^{-2} (ref. 6). Thus as the planet gets warmer, it shifts from a solar-energy-dominated regime to a regime in which the greenhouse effect becomes comparable to the solar absorption. The extreme example of this convective response to warming is Venus, with a convectively driven deep troposphere of ~60 km, overcast conditions and a high planetary albedo of 80% (compared with 30% for Earth). As a result, although Venus receives twice as much solar radiation as Earth at the top of the atmosphere, the absorbed solar radiation is smaller (~150 W m^{-2}). But in spite of this brightening effect, the planet is hotter (750 K) because its atmospheric greenhouse effect exceeds 17,000 W m^{-2}.

The feedback between convection, greenhouse effect and planetary brightness limits the temperatures in the warmest regions of the Earth to less than 305 K. What is the implication of this negative feedback if its validity extends to a perturbed atmosphere? It would take more than an order-of-magnitude increase in atmospheric CO_2 to increase the maximum SST by a few degrees, in spite of a significant warming outside the equatorial regions. In this regard, the present hypothesis departs considerably from modern general circulation models. It is encouraging, however, that some model studies[1,16] are finding a marked feedback in the thermodynamic link between SST and cirrus properties. More significantly, a recent coupled ocean-atmosphere/general circulation model study[28] has suggested that the interaction between SST and solar radiation at the ocean surface is an important negative feedback in the El Niño dynamics. This result was also indicated qualitatively by earlier studies[15,38]. For the hypothesis to become a validated theory, we need to establish the link between convection dynamics, large-scale moisture convergence into the warm regions and the brightness of those regions. Three-dimensional climate models in conjunction with detailed field and satellite observations can provide that link, but first the models must pass the test posed by the El Niño observations. □

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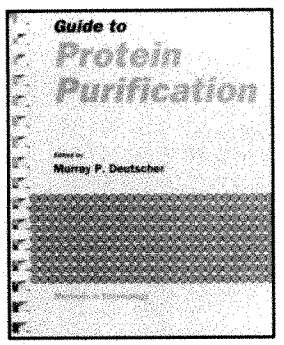

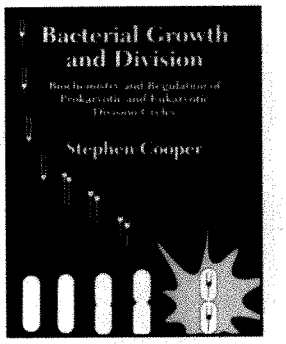

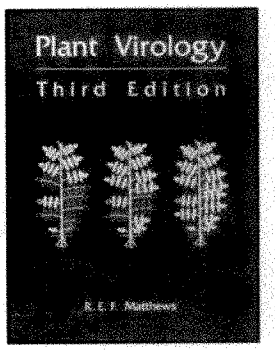

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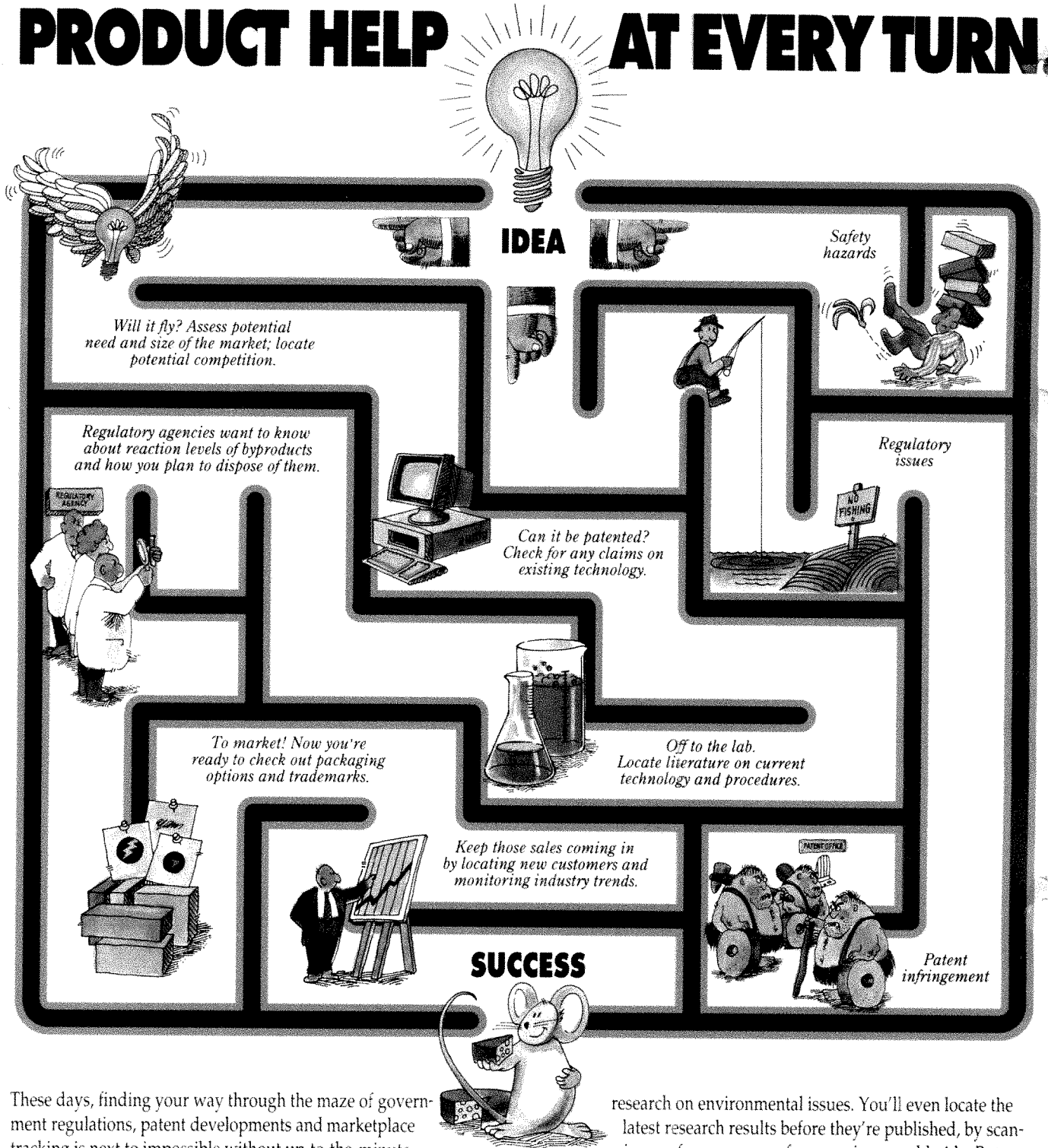

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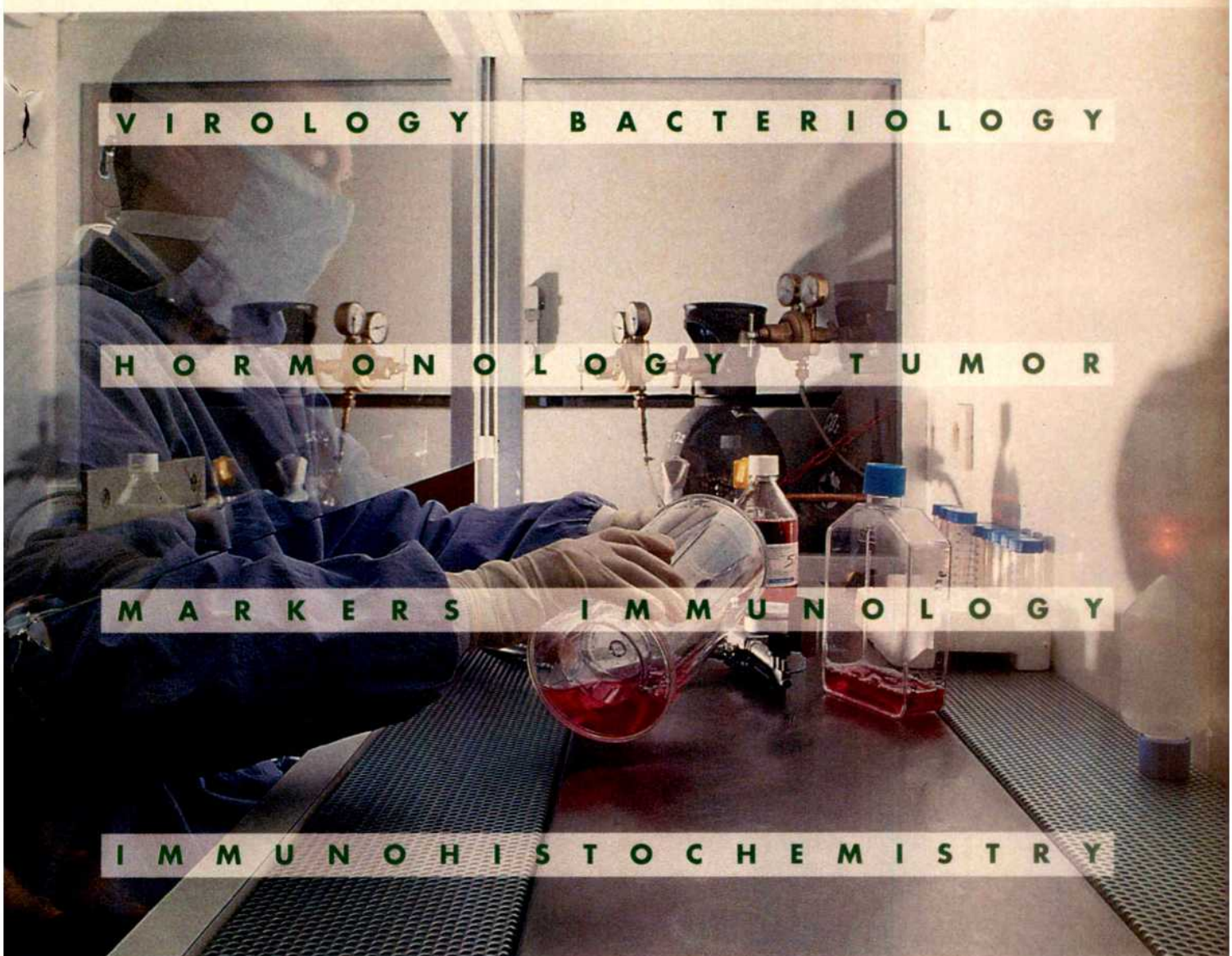

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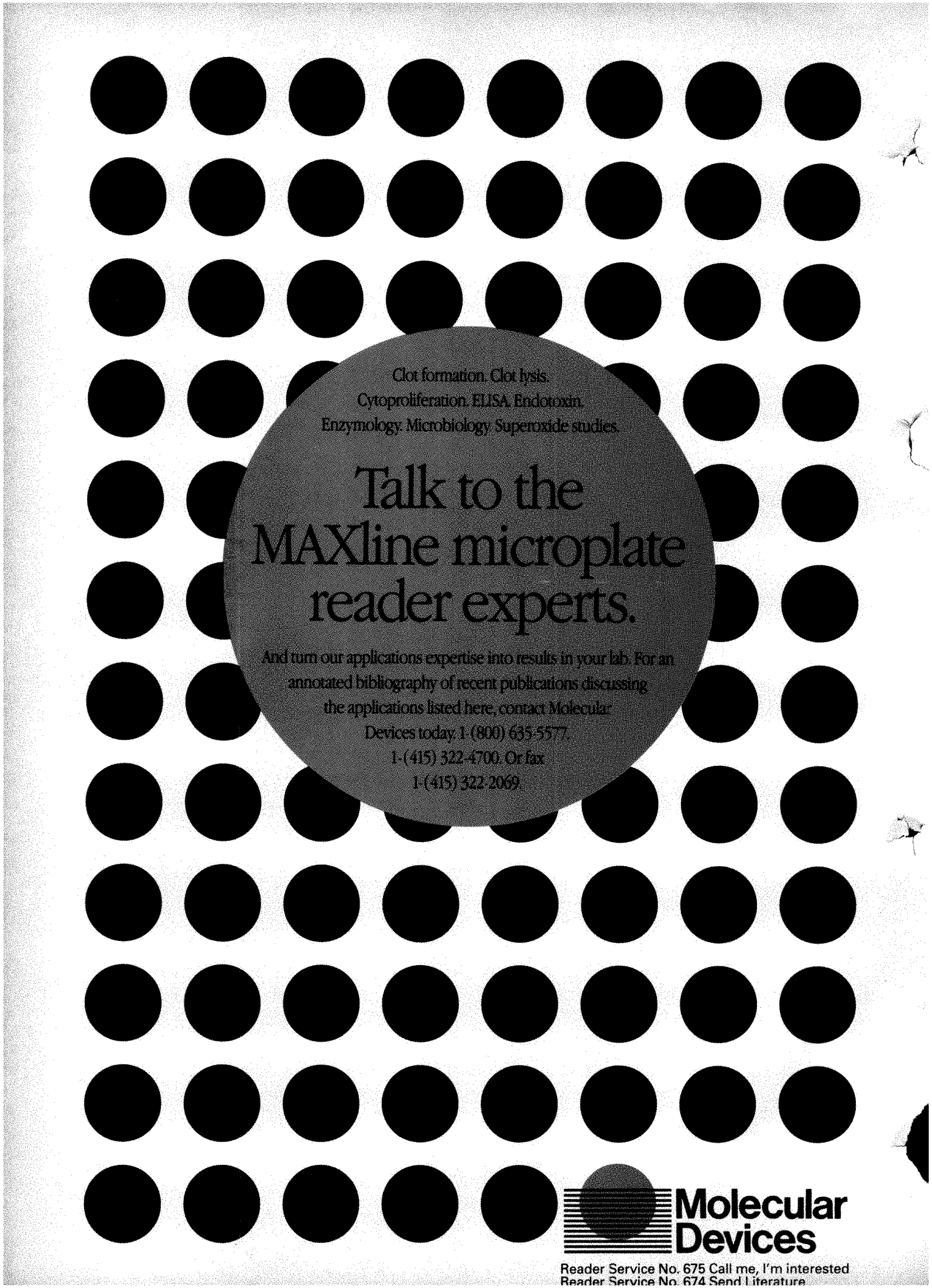
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Pathway of phosphatidylinositol(3,4,5)-trisphosphate synthesis in activated neutrophils

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Neutrophils activated by the formyl peptide f-Met-LeuPhe transiently accumulate a small subset of highly polar inositol lipids. A similar family of lipids also appear in many other cells in response to a range of growth factors and activated oncogenes, and are presumed to be the direct or indirect products of 3-phosphatidylinositol kinase. The structures of these lipids are shown to be phosphatidylinositol 3-phosphate, phosphatidylinositol-(3,4)bisphosphate and phosphatidylinositol-(3,4,5)trisphosphate, and we present evidence that in intact neutrophils a phosphatidyl-inositol-(4,5)bisphosphate-3-kinase seems to be the focal point through which agonists stimulate the formation of 3-phosphorylated inositol lipids.

SEVERAL extracellular agonists and growth factors stimulate the rapid transient formation of at least two highly polar inositol phospholipids[1-6], neither of which seem to possess the conventional head-group structure of phosphatidylinositol(4,5)-bisphosphate, the ubiquitous precursor of the second messenger Ins(1,4,5)P_3 (ref. 7). The molecules are of interest not only because they represent a new class of compounds, rapidly generated on cell activation, but also because their synthesis correlates, possibly causally, with the agonist-driven entry of some cells into the mitotic cycle[8,9]. Moreover, an enzyme activity that can synthesize chromatographically similar compounds *in vitro*[3] is translocated rapidly in the cell when activated by many growth factor receptors[3,4,10] and on transformation of cells by oncogenes[11-13]. Although the structures of these new lipids are likely to be the 3-phosphorylated analogues of PtdIns(4)P and PtdIns(4,5)P_2[13-15], this has not been directly established, and moreover recent reviews[14,15] have concluded that very different pathways are responsible for their appearance in agonist-stimulated cells (Fig. 5).

Polyphosphoinositides in human neutrophils

Traynor-Kaplan *et al.*[1,2] showed that in human neutrophils *N*-formylmethionylleucylphenylalanine (FMLP) causes the rapid accumulation of two novel inositol phospholipids which are likely to be a PtdInsP_2 (although not PtdIns(4,5)P_2) and a PtdInsP_3. We have similarly labelled intact human neutrophils with [^{3}H]Ins and/or $^{32}P_i$ *in vitro*, and then analysed their head-group structures and metabolism. Two PtdInsPs, two PtdInsP_2s and a PtdInsP_3 were characterized. The structures of three polyphosphoinositol lipids, phosphatidylinositol 4-phosphate (PtdIns(4)P), phosphatidylinositol 3-phosphate (PtdIns(3)P) and phosphatidylinositol(4,5)-bisphosphate (PtdIns(4,5)P_2), from bovine brain[16] and cultured astrocytoma cells[17], have been established, and are confirmed here for human neutrophils (data not shown). The two remaining lipids (which on deacylation yielded peaks 1 and 3 in Fig. 1) had the structures of D-phosphatidylinositol(3,4)-bisphosphate (PtdIns(3,4)P_2) and D-phosphatidylinositol(3,4,5)trisphosphate (PtdIns(3,4,5)P_3). These structures were deduced by enzymatic and chemical dissection and a series of high-resolution chromotographic techniques to resolve the products in the presence of relevant internal standards (Figs 1 and 2).

Synthesis of polyphosphoinositides in neutrophils

The structures suggest, but do not define, the route(s) by which the phospholipids are constructed in the cell (for example PtdIns(3,4,5)P_3 could be derived by the phosphorylation of either PtdIns(3,4)P_2 or PtdIns(4,5)P_2). Clues may be gained about these processes by studying cell extracts capable of catalysing the interconversion of the various metabolites. But such results must be interpreted with caution, because in the intact cell a huge variety of compartmentation factors or 'environmental' conditions not mimicked *in vitro* may alter the distribution, pattern or even direction of flows of intermediates through the pathways.

Using intact human neutrophils, we have analysed the relative rates at which $^{32}P_i$ is incorporated into each of the phosphate groups of the polyphosphoinositides *in vitro*. $^{32}P_i$ will be incorporated most rapidly into a polyphosphoinositide if it is the last phosphate moiety to be added and progressively less rapidly earlier in the pathway (assuming each of the phosphate moieties comes from the same precursor, for example the γ-phosphate of ATP). After prolonged periods of incubation, when the sytem approaches equilibrium, these phosphate-specific differences in labelling vanish (for a more detailed explanation of this strategy, see refs 18, 19).

We labelled the cells with $^{32}P_i$ for 18 min and quenched them after 8 seconds in the presence of FMLP. This allowed the label to enter the precursors but was sufficiently brief that the specific radioactivities of the γ-phosphate of ATP and the phosphate groups of the polyphosphoinositides, and also the concentration of PtdIns(3,4,5)P_3, were still increasing rapidly (and moreover that the 5-phosphate of PtdIns(4,5)P_2 was significantly more radioactive than the 4-phosphate; L.R.S., unpublished data, and see Table 1 and Fig. 3). The distribution of ^{32}P within the various polyphosphoinositide species was then determined as described in the legend to Table 1.

Of the total ^{32}P in PtdIns(4,5)P_2, 0.2, 43 and 57% were located in the 1- (phosphodiester), 4- and 5- (phosphomonoester) phosphates, respectively. Similarly, essentially all of the total ^{32}P in PtdIns4P was in the 4-phosphate. These results confirm the metabolic map defined by Brockerhoff and Ballou[18] (PtdIns⇄PtdIns(4)P⇄PtdIns(4,5)P_2) and represent the only direct evidence that in an intact hormone-sensitive cell, PtdIns(4,5)P_2 is synthesized using PtdIns(4)P.

A similar analysis of PtdIns(3,4,5)P_3 (Table 1) revealed that the 3-phosphate contained the largest share of ^{32}P, suggesting that this lipid had been formed by the phosphorylation of PtdIns(4,5)P_2. The distribution of ^{32}P among the 1-,4- and 5-phosphates of PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 was consistent with the synthesis of PtdIns(3,4,5)P_3 beginning with

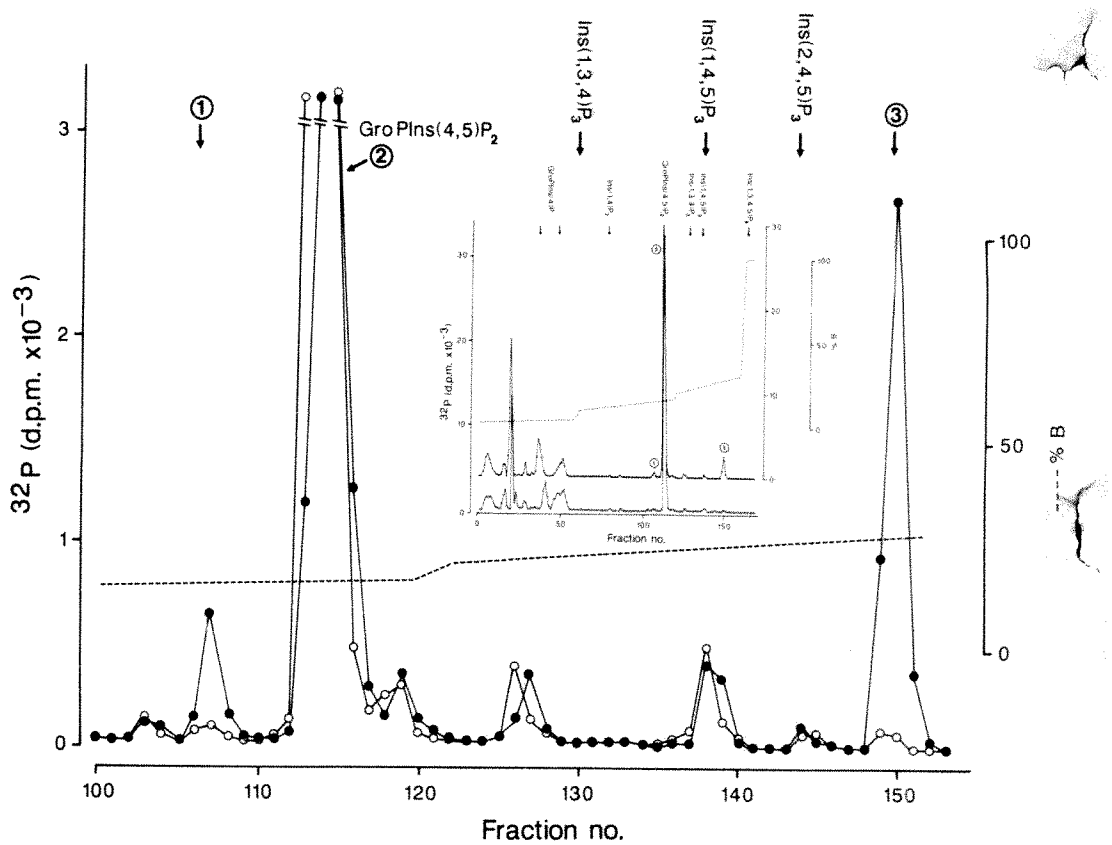

FIG. 1 Anion-exchange HPLC separation of the deacylation products derived from lipid extracts of either control (○) or FMLP-stimulated (●) ^{32}P-labelled (8 s) human neutrophils. Inset figure shows the entire elution profile. The elution times of internal ^{3}H-labelled standards are shown. The traces are typical of over 60 separations. Internal GroPIns(4)P standards usually eluted as two poorly defined peaks (see below and the legend to Fig. 3).

METHODS. A partisphere 5-SAX column was eluted at 1 ml min^{-1} with a gradient of 2.5 M NaH_2PO_4 (pH 3.8 with NaOH (%B)); fractions were collected every 0.5 min. Human neutrophils were labelled *in vitro* with either (1) ^{3}H-Ins or (2) $^{32}P_i$ as follows. (1) Three separate 5-ml incubations, each containing 5×10^7 cells per ml (see legend to Fig. 3) and 1 mCi ml^{-1} ^{3}H-Ins (Amersham; 18 Ci mmol^{-1}) in RPMI medium (without Ins; Gibco) supplemented with 1% (v/v) penicillin and streptomycin (Flow Labs), 10% (v/v) 190 mM HEPES (pH 7.2, 37 °C with NaOH), 1% (v/v) 200 mM EGTA (pH 7.2, 37 °C with NaOH) and 0.5% (w/v) BSA. The incubations were shaken in an orbital mixer at 200 r.p.m. and 37 °C for 5.5 h, then stimulated with FMLP (1 μM final concentration) for 15 s before quenching the cells and extracting their phospholipids as described in the legend to Fig. 3*b*. To one of the three incubations, 0.1 μCi of ^{32}P-PtdIns(4,5)P_2, prepared as described[31], was added. (2) A 1.3-ml incubation containing 5×10^7 cells per ml and 20 mCi ml^{-1} $^{32}P_i$ (PBS-43, Amersham) as described in the legend to Fig. 3, except that the cells were labelled for only 18 min before being stimulated with FMLP (1 μM) for 8 s with the original $^{32}P_i$ still present in the incubation. The cells were quenched and the phospholipids extracted as described above. The lipid extracts were deacylated and resolved by anion-exchange HPLC as described above and in Fig. 3. From the labelling scheme (1) three ^{3}H-labelled peaks were collected (their $^{32}P_i$-labelled counterparts are illustrated) which eluted as follows. 1, A peak just before the internal ^{32}P-GroPIns(4,5)P_2 (derived from the ^{32}P-PtdIns(4,5)P_2 spiked into one of the three parallel extracts) which was agonist-sensitive and contained no ^{32}P. 2, The major ^{3}H-Ins-labelled peak that co-migrated with the internal ^{32}P-GroPIns(4,5)P_2 generated by deacylation of the added ^{32}P-PtdIns(4,5)P_2. 3, A peak that ran at the time expected for the agonist-sensitive 'GroPInsP_3' studied (and previously described[1,2]), which contained no ^{32}P from the internal ^{32}P-PtdIns(4,5)P_2 and co-migrated with the water-soluble ^{32}P-labelled deacylation product of the ^{32}P-lipid formed in assays containing PtdIns(4,5)P_2, γ-^{32}P-ATP and proteins immunoprecipitated with an anti-phosphotyrosine monoclonal antibody (ICN) from PDGF-stimulated Swiss 3T3 cells[32] (data not shown). These three peaks were isolated, pooled (from all three samples in the cases of peaks 1 and 3 and only from those two ^{3}H-labelled extracts which were not spiked with ^{32}P-PtdIns(4,5)P_2 in the case of 3) and desalted[18]. The total ^{3}H d.p.m. recovered in compounds 1, 2 and 3 were 97,700 d.p.m., 2,148,250 d.p.m. and 210,000 d.p.m., respectively. From the labelling scheme (2), three ^{32}P-labelled peaks showing the same properties as the three ^{3}H-Ins-labelled peaks defined above (and which contained 26,487, 773,934 and 47,558 d.p.m. of ^{32}P, respectively) were similarly isolated from the ^{32}P-labelled extract. Aliquots (50–70%) from each of the three ^{3}H-Ins-labelled peaks were mixed with all of these corresponding ^{32}P-labelled peaks (except in the case of ^{32}P-GroPIns(4,5)P_2, of which only 20% was used) then re-chromatographed on an anion-exchange HPLC column as described. In each case the ^{32}P and ^{3}H co-eluted as single peaks at the same time as they had originally, and these were collected and desalted before further analysis (Table 1). Aliquots of the residual ^{3}H-labelled compounds were analysed as follows to determine their structure. Peaks 1, 2 and 3 all gave ^{3}H-GroPIns when dephosphorylated with alkaline phosphatase[17]. Peak 1 was deglycerated[17], the product co-chromatographed with internal ^{32}P-Ins(1,3,4)P_3 but not with internal ^{32}P-Ins(1,2,4)P_3 (Fig. 2*d*) and on periodate oxidation, reduction and dephosphorylation[16] (PRD) yielded ^{3}H-L-altritol (refs 33, 34). This compound was therefore Ins(1,3,4)P_3 and as its 1-phosphate and the diester phosphate of peak 1 are identical (they both contained <0.5% of the total ^{32}P in their respective molecules, see Table 1 and ref. 17), peak 1 must be GroPIns(3,4)P_2. Deglyceration of peak 2 yielded a compound which co-chromatographed with internal ^{32}P-Ins(1,4,5)P_3 but not internal ^{32}P-Ins(1,4,6)P_3 (similar to Fig. 2*c*) and upon PRD yielded ^{3}H-D-iditol. This compound is therefore Ins(1,4,5)P_3 and by the same logic as above (Table 1) its 1-phosphate and the diester phosphate of 2 are equivalent, so peak 2 is GroPIns(4,5)P_2. Peak 3 was deglycerated to an InsP_4 whose chromatographic properties are illustrated in Fig. 2*a, b*, and upon PRD gave ^{3}H-inositol (unless pretreated with 1 M HCl at 80 °C for 8 min, in which case it gave ^{3}H-glucitol and ^{3}H-inositol). The InsP_4 was also dephosphorylated with human erythrocyte plasma membranes (5-ml assays containing 4 mg ml^{-1} erythrocyte protein, 12.5 mM HEPES, 1.0 mM EGTA, 1 mg ml^{-1} BSA, pH 7.0, 37 °C and 500 d.p.m. of ^{32}P-Ins(1,3,4,5)P_4) in the presence of either 10 mM $MgCl_2$ for 30 min or of 5 mM EDTA for 60 min. Under both sets of conditions the ^{3}H-InsP_4 was metabolized at an identical rate to the internal ^{32}P-Ins(1,3,4,5)P_4. The former conditions gave an ^{3}H-InsP_3 which was identified as ^{3}H-Ins(1,3,4)P_3 by the same criteria as above (similar to Fig. 2*d*) and the latter, ^{3}H-Ins(1,4,5)P_3, again identified by the same criteria as above (Fig. 2*c*). As with peaks 1 and 2 above, the data in Table 1 establish that the 1-phosphate of the Ins(1,3,4,5)P_4 derived from peak 3 was the only phosphate moiety that contained an equivalent amount of ^{32}P to the phosphodiester phosphate of 3 and hence peak 3 is GroPIns(3,4,5)P_3.

TABLE 1 ^{32}P distribution in polyphosphoinositides extracted from FMLP—stimulated neutrophils

Phospholipid	Phosphodiester phosphate	Identity of individual phosphates (% of total ^{32}P) 1	3	4	5	100% values total d.p.m. ^{3}H	^{32}P
PtdIns(3)P	0.0	0.2	100.7			5,508	1,906
PtdIns(4)P	0.2	0.4		99.6		95,020	50,125
PtdIns(3,4)P_2	0.3	0.5 ± 0.67 (3)	62.0 ± 0.3 (2)	37.8 ± 1.2 (2)		54,429	22,463
PtdIns(4,5)P_2	0.0	0.2		43.2	56.6	1,565,731	88,135
PtdIns(3,4,5)P_3	0.2	0.04 ± 0.8 (4)	44.8 ± 0.7 (5)	24.9 ± 0.8 (4)	30.4 ± 0.6 (2)	110,991	38,858

Human neutrophils were labelled with $^{32}P_i$ for 18 min, stimulated with FMLP for 8 s, quenched and a phospholipid extract deacylated as described in the legend to Fig. 1 (the glycerophosphoinositol phosphates GroPIns(3)P and GroPIns(4)P analysed in these experiments were obtained from samples prepared in parallel to those described in Fig. 1, except that they were purified by TLC before deacylation; see legend to Fig. 3). The intramolecular distribution of ^{32}P in each of the polyphosphoinositides was determined as described below and is shown as the percentage of the total ^{32}P in a particular lipid found in each of its constituent phosphate moieties. Where more than one independent estimate (by analysis of different degradation products, see below) of these values was obtained (number defined in brackets), then the figures are given as means ± the maximum range of the data used to define that mean. The total ^{3}H and ^{32}P d.p.m. which defined the 100% values for each of the lipids are indicated in the table. The overall strategy and liquid scintillation counting techniques used were as described in ref. 18. The starting compounds (dual-labelled with ^{3}H in the inositol moiety, and $^{32}P_i$) were systematically dephosphorylated, the products at each stage were purified by HPLC, fully structurally characterized and their ^{3}H and ^{32}P content quantified. The ^{3}H recovered served as a measure of the yield and the ^{32}P content defined the total ^{32}P contained by the phosphates present in that particular metabolite. The ^{32}P content of individual phosphate moieties was determined as differences in the molar ^{32}P content of the various metabolites, or directly when an inositol monophosphate was considered. The ^{32}P content of the phosphodiester phosphate of each compound was defined by incubating their respective glycerophosphoinositol phosphates with alkaline phosphatase[17]. This showed that the phosphodiester phosphates in the glycerophosphoinositol phosphates and the 1-phosphates in the inositol phosphates derived from them were structurally equivalent (see Fig. 1). The remaining samples were converted to their corresponding inositol phosphates[17] (this process had no effect on the $^{3}H/^{32}P$ content of any of the lipid derivatives being analysed) and these were then enzymatically dephosphorylated in various ways. Ins(1,3,4,5)P_4 samples were metabolized with human erythrocyte plasma membranes either in the presence of Mg^{2+} or EDTA (see legend to Fig. 1) to form Ins(1,3,4)P_3 and Ins(1,4,5)P_3, respectively. Ins(1,3,4)P_3 samples (either derived from GroPIns(3,4)P_2 or via Ins(1,3,4,5)P_4 from GroPIns(3,4,5)P_3) were metabolized with (1) Ins(1,4)P_2–Ins(1,3,4)P_3 1-phosphate phosphatase (purified 800-fold from bovine brain supernatant[48] by ammonium sulphate fractionation and anion-exchange chromatography on a Mono Q FLPC column[49,50]) to form P_i and Ins(3,4)P_2, or (2) a bovine brain supernatant (0.55 mg ml^{-1} final protein in the presence of 5 mM EDTA, 25 mM HEPES, 1 mM EGTA, pH 7.0, 37 °C) to form P_i, Ins(1)P and Ins(1,3)P_2. Ins(1,4,5)P_3 (either derived from GroPIns(4,5)P_2 or via Ins(1,3,4,5)P_4 from GroPIns(3,4,5)P_3) was metabolized with either (1) human erythrocyte ghosts in the presence of Mg^{2+} (see legend to Fig. 1) to form Ins(1,4)P_2, or (2) a bovine brain supernatant (2 mg ml^{-1} final protein in the presence of 5 mM $MgCl_2$, 5 mM Ins(2)P, 25 mM HEPES, 1 mM EGTA, pH 7.0, 37 °C) to form predominantly Ins(4)P (over 90% of the ^{3}H-labelled products was recovered in Ins(4)P). Ins(1,3)P_2 (derived from GroIns(3)P) was converted to Ins(1)P and P_i as described[17]. Ins(1,4)P_2 (derived from GroPIns(4)P) was metabolized to Ins(4)P under the conditions defined for Ins(1,4,5)P_3 above. Ins(3,4)P_2 (derived from GroPIns(3,4)P_2 through Ins(1,3,4)P_3 or from GroPIns(3,4,5)P_3 through Ins(1,3,4,5)P_4 and Ins(1,3,4)P_3) was metabolized with bovine brain supernatant (under the conditions defined for the metabolism of Ins(1,3,4)P_3 above); the only significant products were Ins(3)P and P_i.

PtdIns and progressing through the intermediates PtdIns(4)P and PtdIns(4,5)P_2. The data are neither consistent with the synthesis of PtdIns(3,4,5)P_3 by the 5-phosphorylation of PtdIns(3,4)P_2 (Fig. 5*c* and below), nor with 4-phosphorylation of PtdIns(3,5)P_2 (a lipid recently found in some cells[13,14]). The absence of any detectable PtdInsP_4 in unstimulated cells (or FMLP-stimulated neutrophils) and the rapid accumulation of PtdInsP_3 in response to FMLP (Fig. 3) make it unlikely that PtdIns(3,4,5)P_3 arises by a dephosphorylation.

Analysis of PtdIns(3,4)P_2 extracted from human neutrophils labelled with ^{32}P and stimulated with FMLP (Fig. 1 and Table 1) showed that its 3-phosphate had the highest specific radioactivity and its 1-phosphate the lowest. This implies that PtdIns(3,4)P_2 could be synthesized by the phosphorylation of PtdIns(4)P (but not by the phosphorylation of PtdIns(3)P). This intramolecular distribution of ^{32}P would, however, also arise if PtdIns(3,4)P_2 was formed by the removal of the 5-phosphate from PtdIns(3,4,5)P_3.

A similar situation emerges for the ^{32}P-labelling of PtdIns(4)P (see above) and PtdIns(3)P; both contained over 99% of their total ^{32}P in their monoester phosphate groups. This simple pattern is compatible with the *de novo* synthesis of each of these compounds from PtdIns. But it is possible (given the existence of two forms of PtdInsP_2 and also a PtdInsP_3 containing both 3 and 4 monoester phosphate moieties) that one (or both) of these lipids is formed by dephosphorylation of more highly phosphorylated lipids. We know that PtdIns(4)P can be formed by both mechanisms in reactions catalysed by PtdIns(4)-OH kinase and PtdIns(4,5)P_2 5-phosphatase[20–22]. These alternative explanations for the labelling patterns of PtdIns(3)P and PtdIns(3,4)P_2 are not easily distinguished by experiments using intact cells, and to resolve the issue two strategies can be adopted: (1) measurement of the absolute specific radioactivity of the compounds concerned[18–22] (which requires accurate chemical determinations of the concentrations of these rare compounds); or (2) a detailed analysis of their initial rates of accumulation in response to the stimulus[19].

Early kinetics of FMLP-driven changes

To define the metabolic origins of the PtdIns(3,4)P_2 and PtdIns(3)P generated in human neutrophils, the rates of change in the levels of all of the ^{32}P-inositol phospholipids were determined 0–2 min after the addition of a maximally effective dose of FMLP (Fig. 3). The curves describing the appearance of the family of '3-phosphorylated' inositol lipids show that the production of PtdIns(3)P and PtdIns(3,4)P_2 lags behind that of PtdIns(3,4,5)P_3. The simplest explanation is that PtdIns(3)P and PtdIns(3,4)P_2 are formed by the stepwise dephosphorylation of PtdIns(3,4,5)P_3.

Metabolism in broken cell systems

Although broken cell assays using exogenous phospholipids as substrates should not be used as the only source of data when defining metabolic networks, they can confirm the existence of enzymatic components of a pathway in cells. We sought evidence for the metabolic fate of PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 in human neutrophils by introducing 3-[^{32}P]PtdIns(3,4,5)P_3 and 3-[^{32}P]PtdIns(3,4)P_2 into various broken cell systems under conditions as physiological as possible (Fig. 4). The ^{32}P lipids were incorporated into unilamellar vesicles containing a balance of phospholipids imitating that found in the inner leaflet of the

plasma membrane, and assays were conducted in an ionic environment like that of the cytosol.

When 3-[^{32}P]PtdIns(3,4,5)P_3 was incubated with whole lysates under these conditions, it was rapidly degraded (2.5% per min at a protein concentration of 100 μg ml^{-1} compared with 1.65% per min for PtdIns(4,5)P_2 in the same assays). During these assays, 89% of ^{32}P products were recovered in a PtdInsP_2 which was identified as PtdIns(3,4)P_2 (Fig. 4). The remaining 11% of the recovered ^{32}P-products were in P_i which could mean a maximum of 11% of the metabolism of PtdIns(3,4,5)P_3 was yielding PtdIns(4,5)P_2. But as these lysates can release $^{32}P_i$ from 3-[^{32}P]PtdIns(3,4)P_2 (see below, and L.R.S., unpublished data) it is likely that this is a quantitatively minor degradative route. The highly active PtdIns(3,4,5)P_3-5-phosphomonoesterase(s) responsible for most of the metabolism of PtdIns(3,4,5)P_3 was membrane-associated. Typically, 85–90% of the activity recovered from lysates was particulate and could be differentiated from the PtdIns(4,5)P_2-5-phosphomonoesterase activity in the same membrane preparations by resistance to 10 mM EDTA. The activity (or activities) that released $^{32}P_i$ from 3-[^{32}P]-PtdIns(3,4,5)P_3 was predominantly soluble (92–95% of the activity from fractionated lysates) and was apparently activated by chelation of divalent cations in the assay buffer by EDTA (like a soluble PtdIns(3)P-phosphatase[23]).

We also used 3-[^{32}P]PtdIns(3,4)P_2 as a substrate for neutrophil lysates. Its rate of metabolism was lower (0.22% per min, at a protein concentration of 100 μg ml^{-1}) than that of PtdIns(3,4,5)P_3 and PtdIns(4,5)P_2. Of the total PtdIns(3,4)P_2 phosphatase activity recovered from fractionated lysates, 75–80% was soluble, and in assays conducted with these fractions, 30–40% of the ^{32}P liberated from the substrate was contained in PtdIns(3)P (see legend to Fig. 4). The activity causing the release of $^{32}P_i$ from 3-[^{32}P]PtdIns(3,4)P_2 was soluble and activated by EDTA, as are the analogous activities releasing $^{32}P_i$

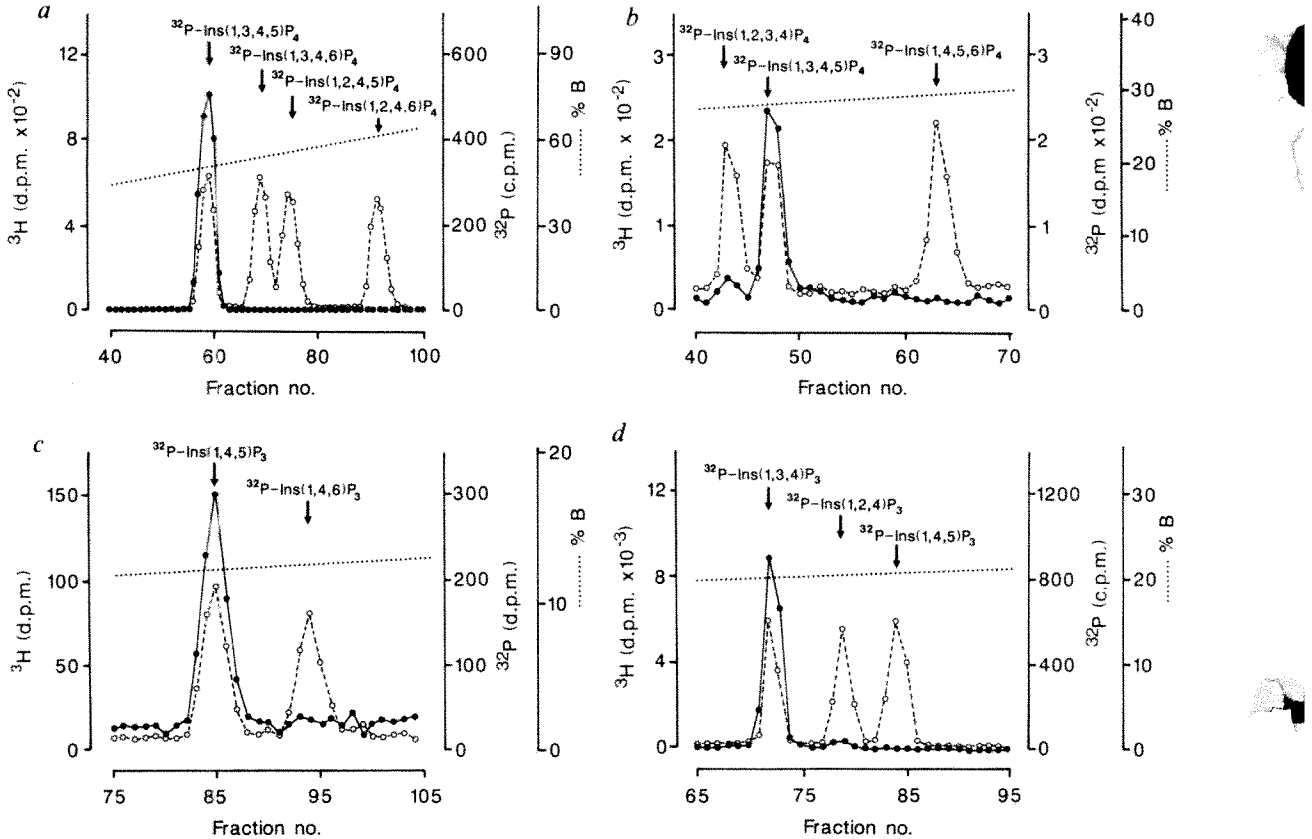

FIG. 2 HPLC analysis of the inositol phosphates derived from peaks 1 2 and 3 in Fig. 1. ○, ^{3}H; ●, ^{32}P. *a*, The ^{3}H-metabolite generated by deglyceration of ^{3}H-GroPInsP$_3$ (peak 3 in Fig. 1) was mixed with ^{32}P-labelled Ins(1,3,4,5)P$_4$, Ins(1,3,4,6)P$_4$, Ins(1,2,4,5)P$_4$ and Ins(1,2,4,6)P$_4$, applied to a Partisphere 5-SAX volume and eluted at 1 ml min^{-1} with a gradient of 2 M NaH_2PO_4 (pH 4.4 with NaOH) (%B). Fractions were collected every 34 s. *b*, An aliquot of the same preparation of deglycerated ^{3}H-GroPInsP$_3$ resolved in *a* was mixed with ^{32}P-labelled Ins(1,2,3,4)P$_4$, Ins(1,3,4,5)P$_4$ and Ins(1,4,5,6)P$_4$ applied to a Partisphere 5-WAX column and eluted at 1 ml min^{-1} with a gradient of 0.5 M $(NH_4)_2HPO_4$ (pH 3.2 with H_3PO_4) (%B). Fractions were collected every 45 s. *c*, The ^{3}H-InsP$_3$ produced by the sequential deglyceration and dephosphorylation (with human erythrocyte plasma membranes in the presence of EDTA) of ^{3}H-GroPInsP$_3$ was mixed with ^{32}P-labelled Ins(1,4,5)P$_3$ (some ^{32}P-Ins(1,4,5)P$_3$ was already present in the samples) and Ins(1,4,6)P$_3$ (see below), applied to a Partisphere 5-SAX HPLC column and eluted at 1 ml min^{-1} with a gradient based on 2 M NaH_2PO_4 (pH 4.4 with NaOH) (%B). Fractions were collected every 35 s. *d*, The ^{3}H-InsP$_3$ derived from the deglyceration of the agonist-sensitive ^{3}H-GroPInsP$_2$ (peak 1 in Fig. 1) was mixed with ^{32}P-labelled Ins(1,3,4)P$_3$, Ins(1,2,4)P$_3$ and Ins(1,4,5)P$_3$ and applied to a Partisphere 5-WAX HPLC column and eluted at 1 ml min^{-1} with a gradient based on 0.5 M $(NH_4)_2HPO_4$ (pH 3.8 with H_3PO_4) (%B). Fractions were collected every 40 s.

METHODS. ^{32}P-labelled Ins(1,4,5,)P$_3$, Ins(1,3,4)P$_3$, Ins(1,3,4,5)P$_4$, Ins(1,3,4,6)-P$_4$ and Ins(3,4,5,6)P$_4$ were prepared as described[18,31,33,34]. ^{32}P-Ins(1,2,3,4)P$_4$ was prepared as the first ^{32}P-InsP$_4$ derived from an extract of ^{32}P-labelled mung beans eluted from a Partisphere 5-SAX HPLC column (it emerges just before internal ^{3}H-Ins(1,3,4,5)P$_4$ when the column is eluted with 1 M NaH_2PO_4, pH 3.8). ^{32}P-Ins(1,2,4,5)P$_4$, ^{32}P-Ins(1,2,4,6)P$_4$ and ^{32}P-Ins(1,2,4)P$_3$ were prepared by treating ^{32}P-Ins(1,3,4,5)P$_4$, ^{32}P-Ins(1,3,4,6)P$_4$ and ^{32}P-Ins(1,3,4)P$_3$ with 1 M HCl (20 min at 80 °C) then purifying the products by HPLC; their identities were established (1) by treating parallel ^{3}H-Ins-labelled extracts with periodate then identifying the polyols generated after reduction and dephosphorylation[16,35] and (2) by comparison of their chromatographic properties with ^{3}H-labelled standards. ^{3}H-Ins(1,2,4)P$_3$ yields ^{3}H-altritol and eluted after Ins(1,3,4)P$_3$ and before Ins(1,4,5)P$_3$. ^{3}H-Ins(1,2,4,5)P$_4$ yielded ^{3}H-Ins and was the earlier eluting of the two products of the acid migration. ^{3}H-Ins(1,2,4,6)P$_4$ was identified as described[23]. ^{32}P-Ins(1,4,6)P$_3$ was prepared by incubating ^{32}P-Ins(1,3,4,6)P$_4$ with human erythrocyte plasma membranes (with 5 mM EDTA, other conditions precisely as described in the legend to Fig. 1); ^{32}P-Ins(1,4,6)P$_3$ constituted 20–30% of the products.

from 3-[^{32}P]-PtdIns(3,4,5)P_3 and 3-[^{32}P]-PtdIns3P (ref. 23, and L.R.S. unpublished data).

Structures and synthesis

It is probable that the structures we have unambiguously derived for the inositol phospholipids found in FMLP-stimulated human neutrophils (and previously for PtdIns(3)P in human astrocytoma cells[17]) will generally be accepted to account for chromatographically similar compounds already identified in cultured cells and other tissues[3-6,24,25]. The metabolic source of these compounds in the intact cell is a more difficult issue. Two different mechanisms are currently proposed to account for the agonist-driven rises in the 3-phosphorylated inositol lipids that occur in PDGF-stimulated smooth muscle[11,13] and thrombin-stimulated platelets[15,26] (Fig. 5*b* and *c*, respectively). Our data are partially consistent with the former model but are not consistent with the latter. The ^{32}P-labelling data suggest that PtdIns(3,4,5)P_3 synthesized in response to FMLP was formed by the 3-phosphorylation of PtdIns(4,5)P_2. But the PtdIns(3,4)P_2 and PtdIns(3)P produced concurrently could have been derived either from the parallel phosphorylation of PtdIns(4)P and/or PtdIns or from the dephosphorylation of PtdIns(3,4,5)P_3 or PtdIns(3,4)P_2. We suggest that the PtdIns(3,4)P_2 and PtdIns(3)P are derived from the sequential dephosphorylation of PtdIns(3,4,5)P_3 because of (1) the kinetics of the appearance of PtdIns(3)P and PtdIns(3,4)P_2 in FMLP-stimulated neutrophils (Fig. 3) and (2) the activities in broken-neutrophil preparations that can catalyse the appropriate dephosphorylation reactions.

This suggestion differs from the mechanism proposed by Carpenter and Cantley[14] (Fig. 5*b*). Their map was based on experimental evidence that showed that, when the PtdIns-3-OH kinase (translocated and tyrosine phosphorylated in response to PDGF) is assayed *in vitro*, it readily phosphorylates PtdIns and PtdIns(4)P[3,4,13,14,25]. We cannot discount the possibility that direct 3-phosphorylation of PtdIns and PtdIns(4)P occurs in stimulated neutrophils, but the most parsimonious explanation of our data does not require this to occur. Indeed, to explain the formation of PtdIns(3)P and PtdIns(3,4)P_2 by direct phosphorylation of PtdIns and PtdIns(4)P, we would also have to propose that the substrate specificity and/or substrate supply of the agonist-activated 3-kinase is different from that *in vitro*, and moreover that it changes during the first few seconds in its activated state.

The history of the assay of phosphoinositidase C provides a direct precedent for the idea of a discrepancy between the substrate specificity of phospholipid-metabolizing enzymes *in vitro* and *in vivo*. Phosphoinositidases C were originally purified and classified as PtdIns-selective phospholipases C on the basis of their preference for PtdIns *in vitro*. But it was only after these same enzymes were shown to display a marked preference for the polyphosphoinositides *in vivo*[27,28] that it was appreciated

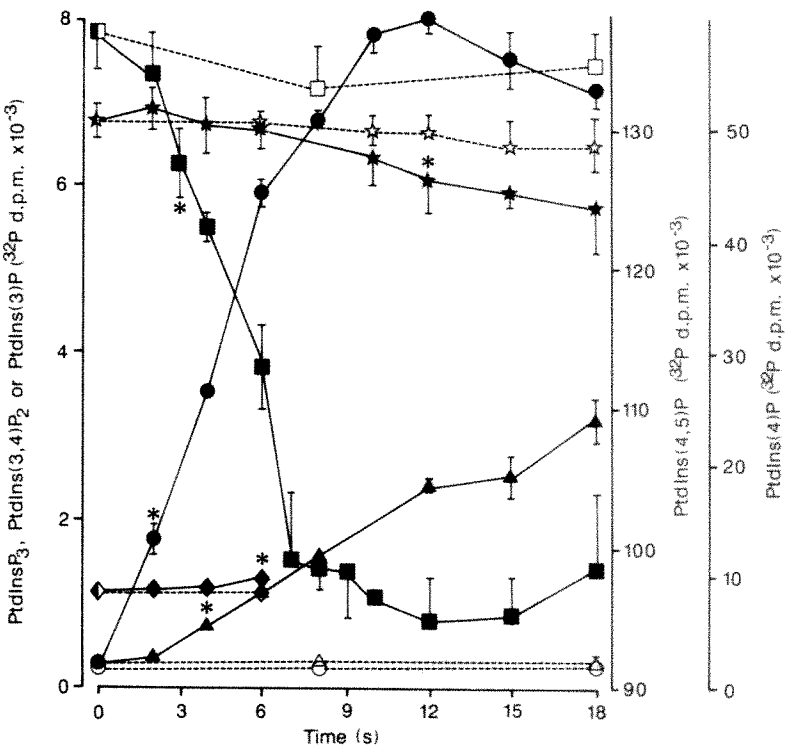

FIG. 3 Rates of change of levels of inositol phospholipids in control (open symbols) and FMLP-stimulated (filled symbols) human neutrophils. PtdInsP$_3$ (circles), PtdIns(3,4)P$_2$ (triangles), PtdIns(3)P (diamonds), PtdIns(4,5)P$_2$ (squares) and PtdIns(4)P (asterisks).

METHODS. Human neutrophils were prepared as described (ref. 36; except that the high-density Percol layer was 1.099 g ml^{-1} and the physiological salt solutions were all as defined in ref. 2) and incubated with ^{32}P$_i$ (5×10^7 cells ml^{-1} 0.4–2.4 mCi ml^{-1} ^{32}P$_i$; PBS-13, Amersham) with no added calcium and with 2 mg ml^{-1} BSA. After 70 min the cells were washed twice with fresh medium, 200–300-μl aliquots were equilibrated (under conditions identical to those in which they were labelled) for 10 min, then mixed with pre-warmed aliquots of medium (as above) either with or without FMLP (final concentration in the cell suspension was 1 μM), quenched and finally a phospholipid extract prepared. The phospholipid extraction protocol was as in refs 1 and 2, except that first, the methanol-HCl solution with which the phospholipid extracts were finally washed also contained 50 mM EDTA, 1 mM cyclohexane-1,2-diamine tetra-acetic acid (CDTA), 1 mM H_3PO_4 and 1 mM myo-inositol; and second, all of the surfaces with which the samples came into contact were polypropylene. Several other extraction protocols were tested[37,38] and none recovered more ^{32}P-PtdInsP$_3$ from a given suspension of cells and some (as in ref. 37) substantially less. To quantify all ^{32}P-phospholipids (except the PtdInsPs; see below) the dry lipid films were deacylated[39] (a ratio of 500 μl deacylating reagent, prepared from monomethylamine gas, to lipid derived from 1.5×10^7 cells was used throughout) and the water-soluble compounds generated were mixed with standards (all prepared either from ^{3}H-Ins-labelled cells or from commercially available standards as described[17,40]) and resolved on an anion-exchange HPLC column (Fig. 1). The ^{32}P compounds resolved were identified by reference to internal ^{3}H-standards and/or the structural analysis shown in the legends to Figs 1 and 2. If neutrophil-derived phospholipid extracts are deacylated[39] directly and analysed by HPLC, then although all other regions of the chromatographic profile elute perfectly reproducibly (both with respect to internal ^{3}H-labelled standards and the endogenous ^{32}P-metabolites), the GroPInsP region eluted at variable times and often as two peaks; when this occurred internally, demonstrably pure ^{3}H-Ins-labelled standards also gave two peaks. This resulted from material derived from the neutrophils that eluted in this region of the HPLC profile, but could be eliminated by purifying the PtdInsPs by TLC before deacylation and HPLC (TLC plates were developed as described[1] except that they were sprayed with 1% potassium oxalate). All PtdIns(4)P and PtdIns(3)P analyses were performed with lipids that had been TLC-purified before being deacylated and finally resolved by anion-exchange HPLC as described above. To quantify the recovery of ^{32}P-PtdInsPs through this process, all extracts were mixed with 10,000 d.p.m. of ^{3}H-PtdIns(4)P (Amersham; recoveries ranged from 82–96%). The data shown are derived from eight experiments and are normalized by reference to the ^{32}P-Pi concentration in the final cell suspensions during the labelling process. Individual points are means ±s.e.m. ($n=3$–10, error bars that fell within the symbols are omitted for clarity). Asterisks indicate the first times at which particular compounds became different from controls ($P<0.05$). Data for ^{32}P-PtdIns(3,4)P_2, ^{32}P-PtdIns(3,4,5)P_3 and ^{32}P-PtdIns(4,5)P_2 contained within those samples that were initially separated on TLC were not included, although they showed exactly the same pattern of change. All of the data points shown are based on measurements of a minimum of 200 d.p.m. ^{32}P above background.

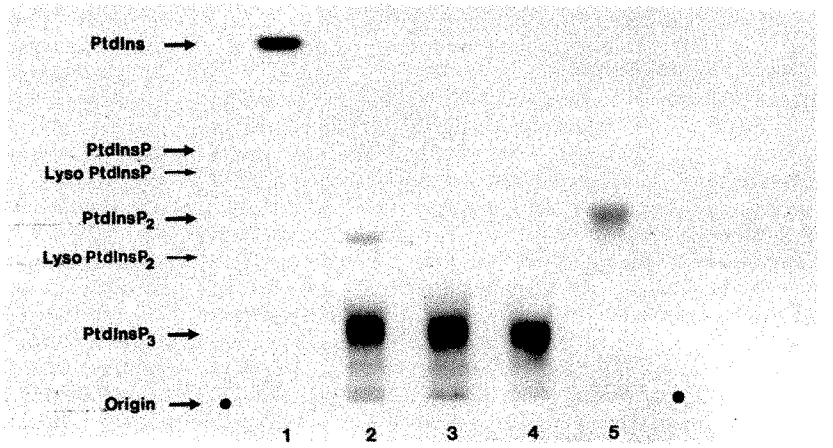

FIG. 4 Metabolism of exogenous phospholipids by cell-free neutrophil preparations. An autoradiogram of a TLC separation of the lipid products formed from 3-[^{32}P]-PtdIns(3,4,5)P_3 by human neutrophil membranes. Lane 1, ^{14}C-PtdIns (Amersham); lane 2, 3-[^{32}P]-PtdIns(3,4,5)P_3 was incubated with human neutrophil membranes (70 μg ml^{-1} protein) for 4 min (8% of the substrate was hydrolysed with no $^{32}P_i$ release); lane 3, as lane 2 but not incubated; lane 4, as lane 2 except incubated with a cytosol fraction (90 μg ml^{-1} protein) from human neutrophils in the presence of 10 mM EDTA for 4 min (8.3% of the substrate was released as $^{32}P_i$); lane 5, ^{32}P-PtdIns(4,5)P_2 standard. The positions to which various standard phospholipids had migrated are marked (they were run on the same plate and detected with iodine vapour).

METHODS. Assay of 3-[^{32}P]-PtdIns(3,4,5)P_3, 3-[^{32}P]-PtdIns(3,4)P_2 and ^{3}H-PtdIns(4,5)P_2 metabolism was as follows. The ^{32}P-lipids were prepared and purified on an amino-propyl HPLC column using 1 M ammonium acetate in chloroform: methanol: H_2O (10:9:1 (v/v); buffer B, see ref. 41). The lipids were stored in buffer B under liquid nitrogen. In some experiments ^{3}H-PtdIns(4,5)P_2 was mixed with the above substrates before adding them to a solution of phospholipids in chloroform (containing by mol per cent, 26.5% PtdIns, 26.5% PtdE, 12% PtdCho, 25.1% PtdSer (Sigma), 2.8% sphingomyelin (Sigma), 2.3% PtdIns(4,5)P_2, prepared as described[42,43]). Unilamellar vesicles were prepared from solutions of phospholipids in chloroform by evaporating the solvent under vacuum then bath-sonicating the lipid films into 20 mM HEPES, 1 mM EGTA, 0.1 mM EDTA (pH 7.2, 25 °C)[32]. Assays of 50 μl contained 15,000 d.p.m. of ^{32}P-lipid (some assays also contained 30,000 d.p.m. of ^{3}H-PtdIns(4,5)P_2), 0.2 mg ml^{-1} unilamellar phospholipid vesicles, 1 mg ml^{-1} BSA, 80 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 20 mM HEPES (pH 7.2, 37 °C) and aliquots of neutrophil lysates, soluble or particulate fractions[44]. Assays were run for 0.5–4.0 min (such that less than 5% of the substrate had been hydrolysed) quenched and phospholipids extracted and either deacylated or resolved by TLC (see above and the legend to Fig. 3). Water-soluble ^{3}H and/or ^{32}P compounds released during these assays were collected from the aqueous phases of the phospholipid extractions, neutralized and resolved by anion-exchange HPLC. The only ^{32}P-labelled product detected was $^{32}P_i$, no water-soluble ^{3}H-metabolites were released during any of the assays. The products of the metabolism of PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 were identified as follows. The ^{32}P-product produced from 3-[^{32}P]-PtdIns(3,4,5)P_3 by neutrophil particulate fractions (lane 2) ran at the trailing edge of the PtdIns(4,5)P_2 used as an internal carrier and was likely to be a PtdInsP$_2$ (on the basis of both the substrate from which it is derived and its chromatographic properties compared to standards, and moreover because it had retained its ^{32}P label, it must contain a 3-phosphate). When samples of the ^{32}P-PtdInsP$_2$ were excised from TLC plates, deacylated and the water-soluble products resolved by HPLC (see legends to Figs 1 and 3) the only detectable ^{32}P-labelled metabolite eluted just prior to internal ^{3}H-GroPIns(4,5)P_2 and at precisely the time expected for GroPIns(3,4)P_2 (data not shown). The ^{32}P-labelled lipid product derived from the dephosphorylation of 3-[^{32}P]-PtdIns(3,4)P_2 by neutrophil cytosolic fractions was identified as PtdIns(3)P on the following grounds: the structure and position of the ^{32}P-label in the substrate from which it was derived, its chromatographic mobility compared to known compounds during TLC (data not shown), and finally, when deacylated the water-soluble head group co-migrated with internal ^{3}H-GroPIns(3)P during anion-exchange HPLC (the ^{3}H standard was prepared from ^{3}H-Ins-labelled astrocytoma cells[17]; data not shown).

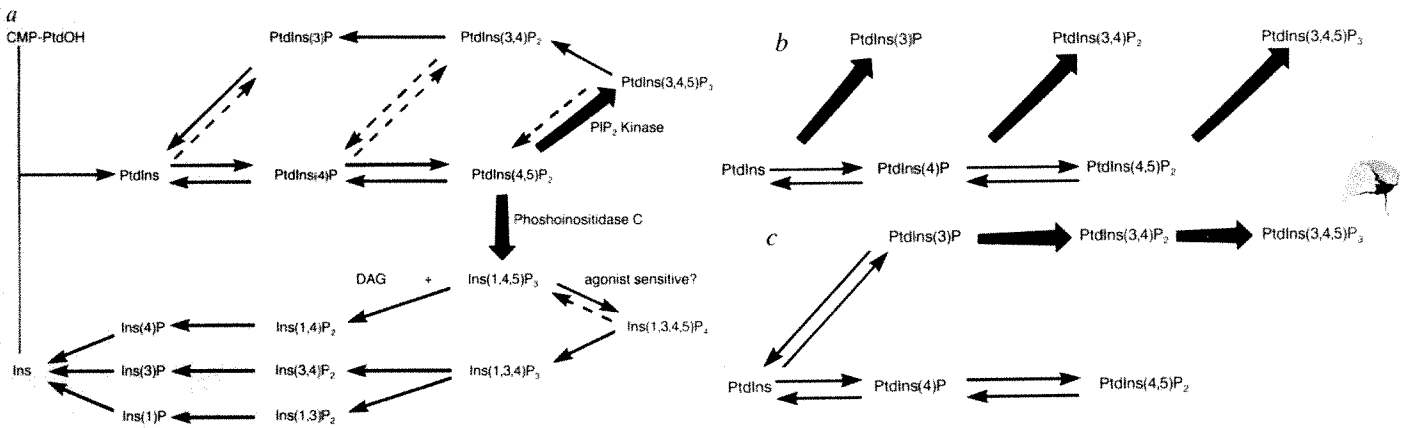

FIG. 5 *a*, Metabolic flow diagram describing FMLP-stimulated polyphosphoinositide metabolism in human neutrophils. FMLP stimulates both a phosphoinositidase C and PtdIns(4,5)P_2-3-OH kinase activity through a pertussis toxin-sensitive GTP-binding protein (possibly Gi2 or Gi3; ref. 45). Phosphoinositidase C liberates the second messengers diacylglycerol (DAG) and Ins(1,4,5)P_3 from PtdIns(4,5)P_2, which are rapidly inactivated by further metabolism[7]. Some recent evidence suggests that Ins(1,4,5)P_3 3-OH kinase may be agonist-regulated[46] and that at least *in vitro* Ins(1,3,4,5)P_4 can be dephosphorylated to Ins(1,4,5)P_3[7]. PtdIns(4,5)P_2 3-OH kinase generates a pulse of PtdIns(3,4,5)P_3 which is rapidly dephosphorylated to yield PtdIns(3,4)P_2 by a predominantly membrane-associated 5-phosphatase that can be distinguished from PtdIns(4,5)P_2 5-phosphatase. A small proportion of PtdIns(3,4,5)P_3 metabolism may be by a 3-phosphatase-yielding PtdIns(4,5)P_2. PtdIns(3,4)P_2 can be dephosphorylated both by soluble and particulate activities, one of the products of which is PtdIns(3)P. The proportion of PtdIns(3,4)P_2 metabolized via PtdIns(3)P was not quantified and theoretically it could be anywhere in the range of 5–100%, hence we have defined the reaction catalysed by the putative PtdIns(3,4)P_2-3-phosphatase activity with a broken arrow. PtdIns(3)P can be dephosphorylated by a soluble PtdIns(3)P phosphomonoesterase activity to yield PtdIns[23]. The reactions by which PtdIns and PtdIns(4)P are phosphorylated *in vitro* to yield PtdIns(3)P and PtdIns(3,4)P_2 have been defined by dashed lines because the events we have defined in FMLP-stimulated neutrophils can be explained without postulating that these activities are expressed in the intact cell. Large solid arrows denote agonist sensitivity. *b*, An alternative scheme for the synthesis of the 3-phosphorylated inositides (as reviewed in ref. 13) which represents the currently held majority view; in this scheme, all three major inositol lipids act as substrates for the 3-kinase(s) such that three concurrent (and presumably roughly equal) paths of synthesis exist. *c*, The route of synthesis proposed for platelets by Majerus *et al.*[15,26], whereby the major substrate of an inositide-3-kinase is PtdIns, and PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 result from successive 4- and 5-phosphorylation of PtdIns(3)P.

that the specificity of phosphoinositidases C *in vitro* are critically dependent on the assay conditions[29].

The model proposed to account for PtdIns(3,4,5)P_3 production in thrombin-stimulated platelets[15,26] (Fig. 5*c*) is incompatible with our data. This could be a result of a cell- or agonist-related difference. The only published information describing the kinetics of this response in the intact thrombin-stimulated platelet clearly shows that PtdInsP_3 levels rose and began to fall before PtdIns(3,4)P_2 concentrations significantly increased[5]. This result would be difficult to reconcile with the synthesis of PtdInsP_3 from PtdIns(3,4)P_2. Clearly, the issue of whether tissues actually differ, or whether experimental protocols account for the difference, remains to be resolved.

Dynamics of PtdIns(3,4,5)P_3 response

The relative flux of material through this pathway and through phosphoinositidase C in stimulated neutrophils can be calculated. We have to assume (1) that the initial rate of agonist-driven depletion of PtdIns(4,5)P_2 represents the sum of the fluxes through phosphoinositidase C and PtdIns(4,5)P_2-3-OH kinase, and (2) that the amounts of radioactivity contained in these lipids in the experiments defined in Fig. 3 reflect their concentrations (allowing for the different complements of monoester phosphates). Assuming these, then 15–20% of the PtdIns(4,5)P_2 that is lost during the first 6 s of stimulation with a maximally effective dose of FMLP flows through PtdIns(4,5)P_2-3-OH kinase. The concentration of PtdIns(4,5)P_2 in resting neutrophils is 50 nmol per ml packed cells (L.R.S., unpublished data). Hence the maximal initial flux through PtdIns(4,5)P_2-3-OH kinase is about 0.34 nmol s^{-1} ml^{-1} packed cells and the concentration of PtdIns(3,4,5)P_3 rises from a basal value of 50 pmol ml^{-1} to a peak value of 2 nmol ml^{-1}. These values suggest intracellular concentrations of PtdInsP_3 of the order of 5 μM (unstimulated) and 200 μM (stimulated), provided that all of this PtdIns(3,4,5)P_3 is present in the inner leaflet of the plasma membrane[30].

The ability of agonists to produce such a localized pulse of a molecule with the structural characteristics of PtdIns(3,4,5)P_3 clearly supports claims that this system may represent a new signalling pathway[13–15] and hence establishes PtdIns(3,4,5)P_3-3-OH kinase as an enzyme of enormous potential interest. □

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Orbital evolution of low-mass X-ray binaries due to radiation driven mass transfer

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A LOW-MASS X-ray binary (LMXB) consists of a compact star, probably a neutron star, accreting mass from a low-mass ($\lesssim 1\ M_\odot$) companion via an accretion disk. Of ~100 known LMXBs in the Galaxy, only four have stable enough X-ray modulations to have allowed the reliable determination of orbital period changes. For these four LMXBs, all of which have $P_{orb} \lesssim 5.6$ h, the measured values[1–4] of $\dot{P}_{orb}/P_{orb}$ disagree markedly with what would be expected for orbital evolution driven by angular momentum loss due to gravitational radiation, possibly supplemented by magnetic braking; the empirically derived timescale for orbital evolution is ~100 times less than expected. On the assumption that the observed period changes are secular, and not due to some longer-term periodic change, I argue here that the observed behaviour of LMXBs can be explained as the result of mass loss from the companion star caused by irradiation of the secondary star and accretion disk by the primary[5]. The typical lifetime of a radiation-driven LMXB is expected to be $\sim 10^6$–10^7 yr. This reduced evolutionary timescale can resolve the statistical discrepancy between the number of binary millisecond pulsars and of their presumed LMXB progenitors if about half of all the LMXBs are radiation-driven[5–7].

The mechanism driving LMXB mass transfer depends greatly upon the nature of the companion star. In models of mass transfer neglecting the effect of radiation from the primary, it is assumed that the companion always fills its Roche lobe. Evolutionary models that neglect the effect of radiation have guided studies of the X-ray emission properties of several individual LMXBs. In the orbital period range of interest here, the mass transfer is driven by the loss of orbital angular momentum due to gravitational radiation (GR)[8,9], possibly supplemented by the 'magnetic braking' effect (MB) if the

sequence companion has a sufficiently large magnetic field and intrinsic mass loss[10-11]. I will refer to this evolutionary model as the 'standard' model. The typical timescale for GR evolution is

$$\tau_{GR} \approx (5\times10^9\ \mathrm{yr})a_{11}^{-4}(M/M_\odot)^{-2}(m/M_\odot)^{-1}(1+m/M)^{-1}$$

where $a_{11} = a/(10^{11}\ \mathrm{cm})$ and a is the orbital radius, and M and m are the masses of the primary and companion star respectively. Magnetic braking may be important in the case of companion stars with radiative cores, in other words main sequence[10,11] and possibly sub-giant stars[12] that lose mass to a wind which is magnetically coupled to the star. Here the relevant timescale[10] is

$$\tau_{MB} \approx (1.8\times10^9\ \mathrm{yr})(B_s/200G)^{-4/3}(R/R_\odot)^{-8/3} \times(\dot{m}_w/10^{-10}\ M_\odot\ \mathrm{yr}^{-1})^{-1/3}(M/1.4M_\odot)(m/0.6M_\odot)$$

where B_s is the surface magnetic field of the companion, R the companion's radius, $R_\odot$ the solar radius, and $\dot{m}_w$ the intrinsic mass loss rate from the companion. The evolutionary timescale, τ_{evol}, predicted by the standard model from the characteristics of the LMXBs in Table 1 is $10^9\ \mathrm{yr} \lesssim \tau_{evol} \lesssim 10^8$ yr.

Measuring $\dot{P}_{orb}$ for a LMXB requires a stable fiducial point in the X-ray light curve. Such a measurement is often difficult because most LMXBs have no eclipses or pulsations. Furthermore, LMXBs have been observed for ~20 yr, and the lower limit for the measurable $\dot{P}_{orb}/P_{orb}$ is $\sim10^{-7}\ \mathrm{yr}^{-1}$. Therefore, no LMXB with measurable $\dot{P}_{orb}/P_{orb}$ is expected if the standard model applies to all LMXBs. But reliable non-zero measurements of the time derivative of the orbital period have recently become available[1-4] for four LMXBs, whose properties are given in Table 1. The measured $\dot{P}_{orb}$s, assumed here to represent secular changes of P_{orb} of these sources, disagree with the standard model. This is particularly the case for 4U1820−30, with $P_{orb} \sim 11$ min[13], for which $\dot{P}_{orb}/P_{orb}$ has the opposite sign to that expected if it underwent binary evolution with a degenerate, Roche-lobe-filling companion (such as GR evolution[14]). In the standard model, binary evolution is given by $\dot{P}_{orb}/P_{orb} = [(1-3n)/2]|\dot{m}|/m$, where the stellar index n is defined by the relation $\mathrm{d}\ln R = n\,\mathrm{d}\ln m$, with R the stellar radius. For a Roche-lobe-filling degenerate companion star fitting the orbital parameters of 4U 1820−30, $n = -\frac{1}{3}$, and therefore $\dot{P}_{orb}/P_{orb}$ should be positive, contrary to observations.

Special physical effects influencing the LMXBs in question might explain the observed $\dot{P}_{orb}/P_{orb}$ in the framework of the standard evolutionary model, but these would need to be different for different sources. They could involve, for example, a fast expansion of the secondary (possibly caused by irradiation[3]), an unusually large surface magnetic field of the companion (as in EXO0748−676), or third-body influence in a triple system or in a globular cluster (as in 4U1820−30; ref. 4).

Here I propose an interpretation of the measured $\dot{P}_{orb}/P_{orb}$ in terms of the radiation-driven (RD) model of LMXB evolution[5,15,16] which could be applicable to a relatively large number of LMXBs[7]. The RD model was applied to Cyg X-3 (ref. 17) to explain what was then considered an exceptionally large $\dot{P}_{orb}/P_{orb}$. We now know of three more LMXBs with a large absolute value of $\dot{P}_{orb}/P_{orb}$, and can assess the more general applicability of RD evolution. Here I aim to show that RD evolution can explain all $\dot{P}_{orb}/P_{orb}$ s yet observed and to identify two classes of RD-LMXBs characterized by different values of the mass loss from the binary.

In a typical LMXB the low-mass companion star is illuminated by a relatively large flux of radiation from the primary star with a spectrum that can drive a strong evaporative wind from its outer atmosphere. Irradiating photons of energy ~0.3–10 keV or of energy ~1 MeV are the most effective in driving the mass outflow[5,7,15,16]. When heating in the outer atmosphere of the companion (or disk) is not balanced by bremsstrahlung, H/He recombination and/or metal-line cooling, an evaporative wind is formed[18]. The radiation-driven mass loss rate can be

TABLE 1 Characteristics of the four LMXBs with measured $\dot{P}_{orb}/P_{orb}$

LMXB	P_{orb} (hr)	$\dot{P}_{orb}/P_{orb}$ (yr^{-1})	Timescale (yr)	Ref.
Cyg X-3	4.82	$+(2.20\pm0.22)\times10^{-6}$	5×10^5	1, 28
X1822−371	5.57	$+(3.40\pm0.94)\times10^{-7}$	2.9×10^6	2
EXO0748−676	3.82	$-(2.02\pm0.28)\times10^{-7}$	5×10^6	3
4U1820−30	0.18	$-(1.08\pm0.19)\times10^{-7}$	10^7	4

written[5,15,16] as $|\dot{m}_{rad}| = 10^{-17} f\hat{L}\ \mathrm{g\,s^{-1}}$, where the dimensionless quantity f depends on the details of energy deposition, radiation transport and hydrodynamics at the base of the corona, and $\hat{L}$ is the effective luminosity incident on the companion, $\hat{L} = \chi L\Delta\Omega$, with $\Delta\Omega$ the effective solid angle subtended by the companion star to its primary, L the luminosity (in $\mathrm{erg\,s^{-1}}$) and χ an attenuation factor which takes into account possible absorption screening, scattering or spectral changes in the disk or corona surrounding the compact star. Previous investigations[18-21] of a radiation-driven mass loss from the first Lagrangian point in the binary system Her X-1 reached the conclusion that the mechanism would not be capable of sustaining a large mass transfer rate given the intensity and flat energy spectrum of Her X-1. But in LMXBs, the energy flux irradiating the companion (~10 times that in Her X-1) and quality of the X-ray spectrum (most of the energy is detected in the soft X-ray band) combine to give an evaporative mass outflow rate of[5,7,16] $\sim10^{18}$–$10^{19}\ \mathrm{g\,s^{-1}}$. Both analytical work and numerical calculations[5] on radiation-driven mass outflows from irradiated companions or outer parts of the accretion disk give a conservative estimate of the range of f for typical LMXBs as $1 \lesssim f \lesssim 10$.

A self-sustained (bootstrap) regime of mass transfer may exist if the accretion-powered illumination of the companion causes mass to be transferred to the neutron star at a rate sufficient to sustain that level of illumination[5,15,16]. If this mechanism of mass transfer occurs in LMXBs, it has two fixed points. For large companion masses, it may sustain a large 'bootstrapped' value of the mass loss rate $\dot{m}^*$. Alternatively, if the companion mass falls below a critical mass, m_c, whose value depends on the nature of the companion as well as on the details of illumination and reprocessing[5], the wind-driven mass loss rate is no longer sufficient to power accretion close to the bootstrapped value, and self-sustained mass transfer ceases completely. The precise value of $\dot{m}^*$ depends on the detailed geometry, hydrodynamics and radiation transport specific to the binary. For the LMXBs of interest here, the expected mass loss is in the range $\sim10^{18}$–$10^{19}\ \mathrm{g\,s^{-1}}$ with $\chi \sim 0.1$ (ref. 22), and the corresponding X-ray luminosity L_X is in the range $\sim10^{37}$–$10^{38}\ \mathrm{erg\,s^{-1}}$ which covers most known LMXBs.

The orbital evolution of an LMXB is determined by angular momentum loss (driven by gravitational radiation or a wind), as well as by mass loss from the companion star. A self-sustained RD mass loss from the companion (or from the outer edge of the disk) markedly changes the standard evolutionary pattern (ref. 23 gives a review of standard orbital evolution). The irradiated companion can transfer mass even if it does not fill its Roche lobe exactly (that is, $\Psi \neq 1$ with $\Psi \equiv R/R_L$, where R_L is the Roche lobe radius of the companion). We obtain

$$\frac{\dot{P}_{orb}}{P_{orb}} = -3\left[1-(1-\beta)\tilde{\alpha}(1+q)-\beta q-\tfrac{1}{3}(1-\beta)\frac{q}{1+q}\right] \times\frac{\dot{m}}{m}+3\left[\left(\frac{\dot{J}}{J}\right)_{GR}+\left(\frac{\dot{J}}{J}\right)_{MR}\right] \quad (1)$$

where $\dot{m}$ is the mass transfer rate, β the fraction of $|\dot{m}|$ which is accreted onto the compact star, $(\dot{J}/J)_{GR}+(\dot{J}/J)_{MB}$ the fractional change of orbital angular momentum from gravitational radiation and magnetic braking, $q = m/M$ the ratio of the companion mass to primary mass M, and $\tilde{\alpha}$ the specific angular

momentum parameter defined by $\delta J = \tilde{\alpha}\delta m(1-\beta)a^2 2\pi/P_{\rm orb}$. The dimensionless parameter $\tilde{\alpha}$ gives the effective angular momentum loss caused by mass loss from the binary. Mass loss from the binary may play an important part in determining the evolution of RD-LMXBs. Complex effects including the geometry, hydrodynamics, final wind velocity, and energy exchange between the outflowing gas and primary star radiation all contribute to the effective value of β. I restrict the analysis here to a few idealized cases which are relevant for the LMXBs of Table 1. For a self-sustained value of the RD mass loss rate $-\dot{m} = |\dot{m}_{\rm rad}| = (10^{-8}\,M_\odot\,{\rm yr}^{-1})\dot{m}_8$, where $\dot{m}_8 = |\dot{m}|/(10^8\,M_\odot\,{\rm yr}^{-1})$, the first term of the righthand side of equation (1), proportional to $\dot{m}/m$ is dominant, and I will neglect the contribution from GR and MB in the following discussion. For degenerate and main-sequence companions, the time derivative of the orbital period may be either positive or negative depending primarily on the value of β for $\tilde{\alpha}$ of order unity. The associated theoretical lifetime can be made to be $\sim 10^7$ yr or less. Figure 1 gives the computed $\dot{P}_{\rm orb}/P_{\rm orb}$ for different values of the mass-loss parameter β and for self-sustained $\dot{m}$ appropriate to the LMXBs of Table 1. The dimensionless parameter $\tilde{\alpha}$ has been fixed to give the specific angular momentum loss at the orbital radius; here I use $\tilde{\alpha} = 1$. The thick horizontal lines in Fig. 1 which correspond to the measured $\dot{P}_{\rm orb}/P_{\rm orb}$ give the mass ranges of the companion obtained from the RD evolutionary model.

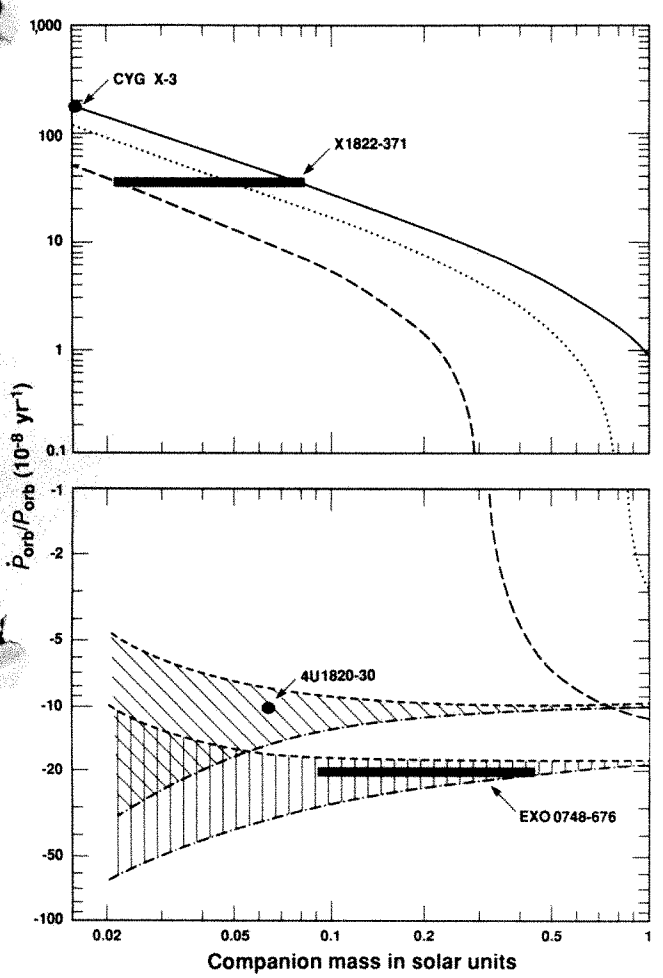

FIG. 1 The quantity $\dot{P}_{\rm orb}/P_{\rm orb}$ computed for the RD evolutionary model, as a function of the mass of the companion for $\tilde{\alpha} = 1$. The upper part of the diagram has a positive $\dot{P}_{\rm orb}/P_{\rm orb}$. The three curves are computed for $\beta = 1$ (solid line), $\beta = 2/3$ (dotted line) and $\beta = 1/3$ (dashed line). The horizontal thick marks, corresponding to the measured $\dot{P}_{\rm orb}/P_{\rm orb}$ for Cyg X-3 and X1822−371, give the mass range for the companion for $\dot{m}_8 = 3$. The lower part of the diagram has a negative $\dot{P}_{\rm orb}/P_{\rm orb}$. The two hatched areas give the allowed range for $\dot{P}_{\rm orb}/P_{\rm orb}$ from the RD evolutionary model with $\dot{m}_8 = 10$ and 20, respectively. The upper dashed curve is for $\beta = 1/10$ and the lower dot-dashed curve for 1/20. The horizontal thick marks, corresponding to the measured $\dot{P}_{\rm orb}/P_{\rm orb}$ for EXO0748−676 and 4U1820−30, give the mass range for the companion stars.

Cyg X-3, with $\dot{P}_{\rm orb}/P_{\rm orb} = +2\times10^{-6}\,{\rm yr}^{-1}$ (ref. 1, 24, 28) fits the evolutionary scenario for a RD-LMXB containing a very low mass white dwarf companion[17]. A strong wind is indeed observed[26,29] in Cyg X-3 and the observed orbital period change agrees with the inferred large mass loss rate in the wind. Even the source X1822−371 has a large and positive rate of change of orbital period[2]. This contrasts with the standard model if the LMXB contains a main sequence companion. As shown in Fig. 1, however, RD-LMXB with a lower main sequence companion may be characterized by a large positive value of $\dot{P}_{\rm orb}/P_{\rm orb}$ if $\dot{m}_8 \gtrsim 3$. Alternatively, X1822−371 may contain a white dwarf companion, and Fig. 1 shows that in this case the mass is probably $m \approx (0.1\,M_\odot)\dot{m}_8$. Both the sign and the magnitude of $\dot{P}_{\rm orb}/P_{\rm orb}$ for Cyg X-3 and X1822−371 suggest a stable RD mass transfer with $\frac{1}{3} \lesssim \beta \lesssim 1$ and $\Psi \lesssim 1$. If both Cyg X−3 and X1822−371 contain a very-low-mass white dwarf, it is natural to consider these systems as progenitors of binaries similar to the eclipsing pulsar[25] PSR1957+20. In the framework of RD evolution, EXO0748−676 and 4U1820−30 are characterized by a large mass loss from the binary with $\beta \sim \frac{1}{10}$. For both low-mass main sequence and degenerate companions, the RD mass transfer is expected to be unstable with $\Psi \gtrsim 1$, in agreement with the large fluctuations in intensity (by a factor of ~5) observed in EXO0748−676 and 4U1820−30. The lower part of Fig. 1 gives the mass range for EXO0748−676 and 4U1820−30 for $\dot{m}_8 \sim 10$ and $\dot{m}_8 \sim 20$, respectively.

The RD model allows two classes of RD-LMXBs, characterized by different values of β. Figure 1 shows that Cyg X-3 and X1822−371 belong to the high-β class, whereas EXO0748−676 and 4U1820−30 belong to the low-β class. New measurements of $\dot{P}_{\rm orb}/P_{\rm orb}$ for additional sources would greatly contribute to the determination of the number and classification of RD-LMXBs. At present $\dot{P}_{\rm orb}/P_{\rm orb}$ has been measured for four out of fifteen LMXBs with $P_{\rm orb} \lesssim 5.5$ h. Of the total sample of ~100 LMXBs only ~36 binaries have well established orbital periods[27]. I urge a complete analysis of selection effects affecting the measurements of $\dot{P}_{\rm orb}/P_{\rm orb}$, using the four LMXBs in Table 1, to obtain an estimate of the true number of RD-LMXBs. □

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The Sun's luminosity over a complete solar cycle

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THE Active Cavity Radiometer Irradiance Monitor (ACRIM I), an instrument carried on NASA's Solar Maximum Mission satellite, measured the Sun's luminosity (total power outflow) from early 1980 to late 1989[1–5]. Here we present the first account of the complete ACRIM I data set, and give evidence confirming our previous suggestion that solar luminosity varies with the 11-year solar cycle[6]. As previously reported, this slow variation closely follows statistical measures of the distribution of magnetic and photospheric features on the Sun's surface[4–8]. But there was an exception to this correlation in the form of a remarkable irradiance excess during 1980, at about the time of the sunspot maximum of solar cycle 21. The linkage, over a whole cycle, of luminosity variation to photospheric activity suggests the existence of an unknown physical mechanism other than the thermal diffusion model that explains luminosity deficits due to sunspots. Luminosity models connecting total irradiance to global indicators of solar activity, such as the equivalent width of the 1,083-nm helium line, are consistent with the gross features of the variability, but fail to account for the 1980 irradiance excess.

Nearly a decade of high-precision monitoring of solar luminosity by the ACRIM I experiment ended with re-entry of the Solar Maximum Mission (SMM) spacecraft in December 1989. Significant solar variability was found on all timescales, ranging from individual samples (1.024 seconds) to the full duration of the observations (9.75 years). These results were the first to demonstrate luminosity fluctuations associated with a wide variety of solar phenomena: sunspots, active regions, oscillations, granulation and the bright network[1–10], a non-uniform, filamentary distribution of excess emission outside active regions.

Here we discuss the most important aspects of the variation in solar luminosity on timescales of a year or more observed by ACRIM I during solar cycles 21 and 22. Such variations must be linked to mechanisms deep within the convection zone or even below, where the source of the solar magnetic cycle is thought to reside. The successes and failures of several empirical models of irradiance will be discussed below[7,8,11].

The results of the ACRIM I experiment, normalized to 1 AU, are shown as daily averages in Fig. 1. The number of independent samples was usually near the maximum available of 10^4 per day during the two periods of precision solar pointing. Daily mean values had an average standard error of 23 parts per million (p.p.m.) of the total flux during the first such period (February to November 1980) and 14 p.p.m. during the second period (after March 1984), reflecting the intrinsic solar variability which was higher during the first of these two experimentally equivalent periods. During the intervening SMM spin-stabilized mode of observation, only ~100 independent samples were acquired per day, resulting in a higher average standard error of the daily means of 76 p.p.m.

The irradiance declined systematically by ~0.1% from launch through mid-1986 to a minimum in near-coincidence with the minimum in solar activity in September 1986. A rapid increase followed, corresponding to the build-up of solar activity in cycle 22.

The general pattern of these results is a variation of the total irradiance in step with the solar magnetic activity cycle, but with considerable short-term fluctuation superimposed on it. The rapid fluctuations on timescales of a few days have been identified with sunspots, and those of a few months, with faculae[1–5]. The data set as a whole strongly suggests a true variation of solar luminosity with the 11-year solar cycle.

It has been suggested previously[7,8,11] that the initial rapid decrease of the total solar irradiance was due to degradation of the sensor during this period. Regression models based on indicators of solar magnetic activity (He 1,083 nm, H Lyman-α and Fraunhofer lines) predict significantly lower values of irradiance than those measured by ACRIM I and an independent experiment, the Nimbus-7/ERB (Earth Radiation Budget)[12,13]. We establish here that (1) the internal calibration of ACRIM I is sound during this interval the good agreement with Nimbus-7/ERB results confirms this calibration; and the mismatch with the regression-based models in 1980 probably indicates the existence of a new feature of the solar cycle that cannot be seen in the previously known indicators.

The 81-day running means of the irradiance measured by ACRIM I and that predicted by a regression model based on the He 1,083-nm flux are shown in Fig. 2. The measured irradiance is corrected for sunspots by the 'photometric sunspot index' (PSI). The model is given as equation (1), where S_{model} is the predicted irradiance (in W m^{-2}) and EW_{He} is the equivalent width of the solar He line at 1,083 nm.

$$S_{\text{model}} = 1{,}364.08 + 0.0636161 \times \text{EW}_{\text{He}} \qquad (1)$$

The plot, in units of percentage variation relative to the means of the time series, shows general agreement between the measured and modelled irradiances except for 1980. The divergence there is significant: the average values for 1980 are 0.093%

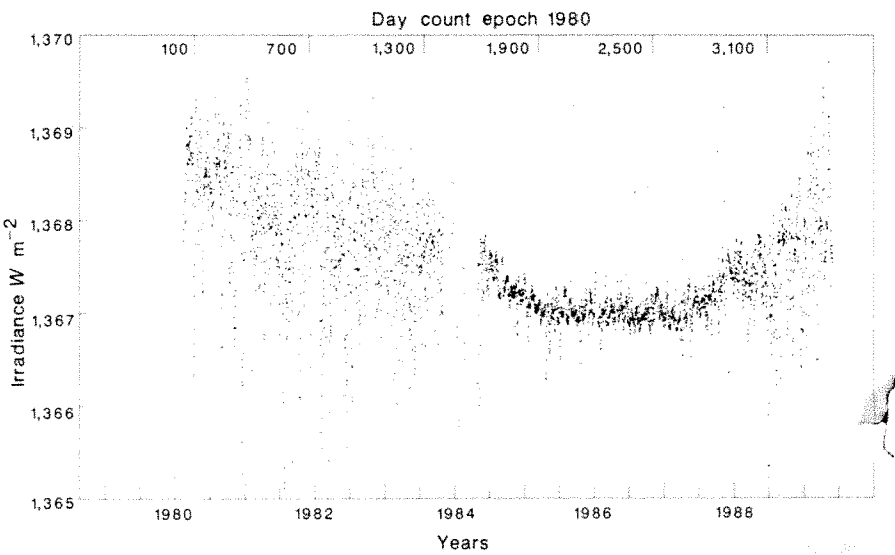

FIG. 1 Total solar irradiance observations by the ACRIM I experiment on the Solar Maximum Mission (SMM). Points are the mean values for a day's observations.

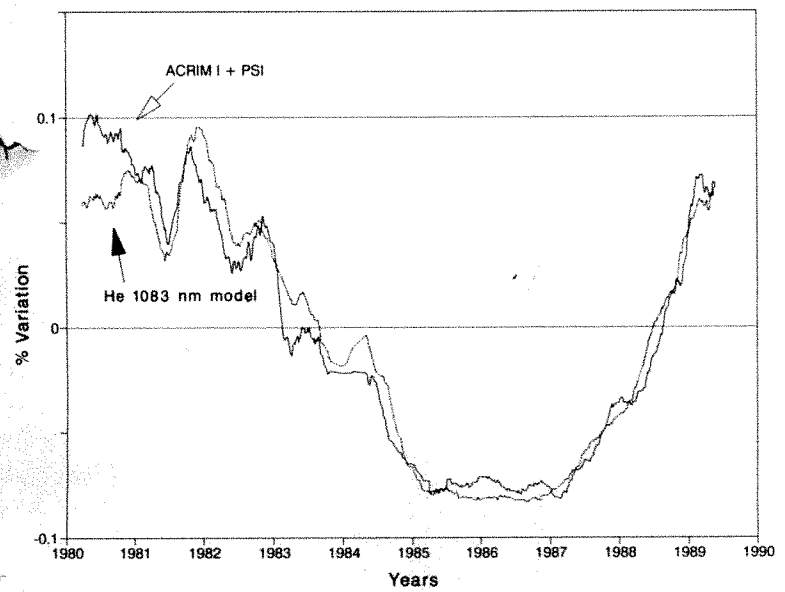

FIG. 2 Percentage variation of 81-day running means for the ACRIM I observations, corrected for sunspot effects by the photometric sunspot index (solid line), and the He 1,083-nm equivalent width regression model of irradiance (dashed line).

for ACRIM I + PSI and 0.063% for the He-1,083 model, an average difference of 0.03%.

Two sources of experimental evidence help us to evaluate the significance of the divergence. The first is another total solar irradiance experiment, the Nimbus 7/ERB mentioned above. We compared the ACRIM I and ERB data after correcting for sunspot effects by using the PSI to remove the irradiance 'sunspot deficit' sensitivity not present in the He 1,083-nm flux[3]. (The ACRIM I and ERB data are sensitive to 'sunspot deficit', whereas the He 1,083-nm flux is not.) The average ACRIM I − ERB residual in 1980 was 0.002%. The irradiance excess of average amplitude 300 p.p.m. in 1980 is therefore 'resolved' by the agreement of ACRIM I and ERB in 1980 to within 20 p.p.m. There is therefore strong experimental evidence for the irradiance excess.

The second source of evidence is an internal calibration system within ACRIM I itself which was sufficient to rule out significant uncorrected degradation at the level of variability detected so far[1]. This calibration is based on comparisons of the three nominally identical active cavity radiometer sensors, which are independently shuttered. The first (A) was used continuously as the solar monitor. Shutters on the second two (B and C) were operated infrequently to calibrate the degradation of the first caused by exposure to the Sun (Fig. 3). Calibration comparisons were made between sensors A and B at roughly monthly intervals, less frequently between all three sensors. The primary sensor's total degradation of 600 p.p.m. over the 9.75-year mission was calibrated with a residual uncertainty of <50 p.p.m. using the ratio of sensors A and C. Eventual degradation of sensor B, the more frequently used calibration reference, can be seen after day 2,500.

A procedure designed to detect and calibrate the initial degradation of ACRIM I's primary sensor[1] found that 85% of the total degradation in 1980 ($\sim$180 p.p.m.) occurred in the first 100 days of observations, from mission days 62 to 163 (Fig. 3). The residual uncertainty of this calibration is better than ± 12 p.p.m., the same order of magnitude as the agreement between ACRIM I and ERB. The 300-p.p.m. 1980 irradiance excess is therefore defined by two independent experimental methods to have an uncertainty of $\leqslant 20$ p.p.m. We believe the variations observed by ACRIM I, including the disputed 1980 irradiance excess, are unambiguously solar in origin.

The ACRIM I data show two principal features of solar luminosity variation on long timescales: the magnetic cycle itself and the irradiance excess of 1980. From the strict standpoint of time-series analysis, the apparent luminosity variation during the solar cycle must await confirmation by observations during future solar cycles. But solar proxy models may be useful here:

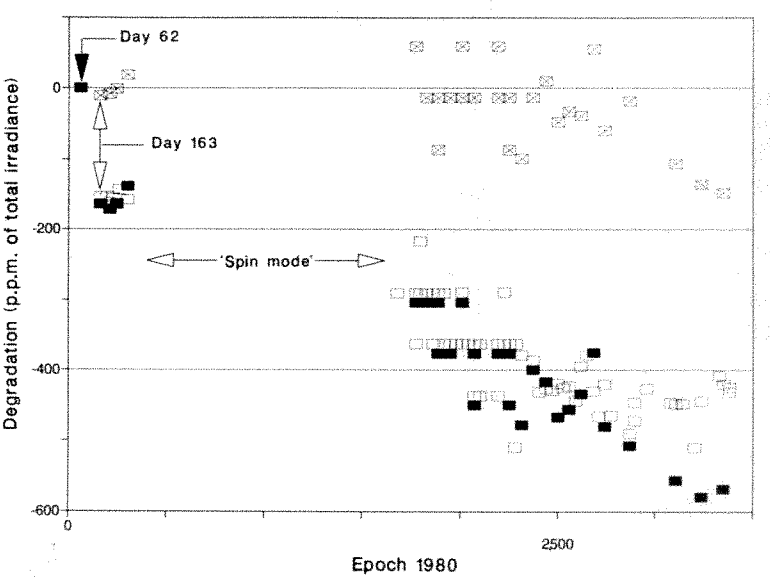

FIG. 3 Results of the ACRIM I degradation calibration experiment plotted as the ratios of its sensors A, B and C. Empty squares are the ratio A/B, solid squares the ratio A/C and crossed squares the ratio B/C. The gap between days 346 and 1,585 results from insufficient sample acquisition during SMM's 'spin mode'.

the gross features of solar cycle behaviour predicted by the He 1,083-nm model matched those of the measured irradiance reasonably well (except for 1980). The data presented here, in this context, indicate that future solar cycles will have similar features which can be detected by sufficiently precise total irradiance observations.

The 1980 irradiance excess is more problematic, as it does not correspond with other indicators of solar activity. The evolution of solar luminosity over the next decade, as chronicled by the ERB, the upcoming and planned satellite missions will be exciting to watch. We should soon have results from the Nimbus-7/ERB experiment with which we can test the prediction made here: of a repetition of the 1980 irradiance excess in 1990–91. □

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A collection of diverse micrometeorites recovered from 100 tonnes of Antarctic blue ice

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STUDIES of meteorites and interplanetary dust particles (IDPs) have provided constraints on the formation and evolution of the Solar System[1], and have identified pre-solar interstellar grains[2–4]. Here we describe a new type of meteoritic material, intermediate in size between meteorites and IDPs. Melting and filtering of ~100 tonnes of blue ice near Cap Prudhomme, Antarctica, yielded ≥7,500 irregular, friable particles and ~1,500 melted spherules, ~100 μm in size, both showing a 'chondritic' composition suggestive of an extraterrestrial origin[5,6]. For the present work, we analysed the composition and texture of 51 irregular particles and 25 spherules. The irregular particles appear to be unmelted, and have similarities with the fine-grained matrix of primitive carbonaceous chondrites, but are extremely diverse in composition. Isotopic analysis of trapped neon confirms an extraterrestrial origin for 16 of 47 irregular particles and 2 of 19 spherules studied, and strongly suggests that they were exposed in space as micrometeoroids. These large Antarctic micrometeorites constitute a new family—or at least a new population—of Solar System objects, in a mass range corresponding to the bulk of extraterrestrial material accreted by the Earth today.

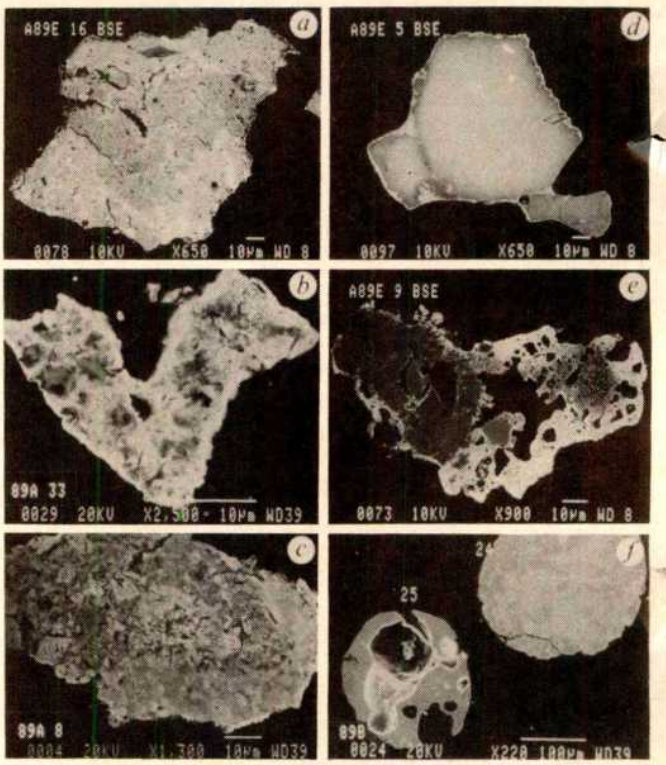

FIG. 1 Scanning electron micrographs of polished sections of ~100 μm sized Antarctic gas rich micrometeorites and spherules. *a*, Very fine-grained unmelted micrometeorite. *b*, Unmelted particle containing observable phyllosilicates and crystals that are too small for individual electron microprobe analysis. *c*, Clast-bearing micrometeorite. *d*, Coarse-grained particle composed of two large olivine crystals, with a small crystal of pyroxene protruding on the lower right corner. *e*, Coarse-grained, partially melted scoria particle with large relict olivines. *f*, Typical vesicular glassy spherule with no relict crystals (left-hand side).

In January 1988, two steam generators delivering pressurized jets of water at 80 °C were used to melt ~40 pockets of ice with volumes of 2–3 m^3 each, ~6 km from the French station of Dumont d'Urville, near Cap Prudhomme, in Antarctica. About 5–10 tonnes of water were pumped and filtered daily on a stack of three stainless steel sieves with openings of 50, 100 and 400 μm. The whole operation yielded ~10 g of dust of diameter ≥50 μm including a total number of ~1,500, ~5,000, and 9 spherules in the 50–100-μm, 100–400-μm and ≥400-μm fraction, respectively. Closer scrutiny in the laboratory gave the ratio of suspected micrometeorites to melted spherules as ≥5 and ~0.3 in the two smaller fractions, respectively.

Seventy apparently unmelted grains were hand-picked from the 50–100-μm fraction, relying on simple criteria such as the particle having a dark grey-to-black colour and irregular shape. Additionally, 30 spherules were selected for study in the same size range. Polished sections were analysed with a scanning electron microscope, equipped with an energy dispersive X-ray spectrometer. Fifty irregular 'stony' grains and all but one of the stony spherules could be classified as 'probably extraterrestrial' relying on the observation that their silicon, magnesium, iron and aluminium contents occurred in roughly chondritic proportions[5,6]. These proportions are similar to those previously obtained for deep sea and Greenland spherules[7,8] containing a high concentration of the cosmogenic nuclides ^{10}Be and ^{26}Al

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and relatively unmelted Greenland micrometeorites[6] containing implanted solar neon. One additional particle has an apparently extraterrestrial metallic Fe-Ni composition. Following optical and scanning electron microscopy, electron microprobe analyses were performed on bulk samples as well as on individual phases, clasts and matrices to determine major and minor element contents (for oxide compositions, see Supplementary Information).

Figure 1 illustrates some textural features of the 50 stony particles. The most common are unmelted particles, composed of fine-grained phyllosilicates with variable amounts of poorly characterized (Fe-Ni) oxide phases, olivines, low-calcium pyroxenes, high-calcium pyroxenes (diopside and fassaite), and rare grains of Fe-Ni metal (Fig. 1*a* to *c*). A few unmelted particles consist mostly of olivine (Fig. 1*d*). There is a range from unmelted particles to partially melted scoria particles, which consist mainly of glass instead of phyllosilicates (Fig. 1*e*). About one-sixth of the 'probably extraterrestrial' particles in the 50-100-μm range are melted spherules (Fig. 1*f*). All selected irregular particles were at least partially covered by a thin rim of magnetite.

Figure 2 compares the bulk major element contents of unmelted and partially melted scoria particles (solid line histogram) with the average composition of primitive carbonaceous chondrites (C1)[9]. From the peaks in elemental ratio distributions, the compositions of micrometeorites look 'approximately chondritic' in Mg, Al, Si and Fe, but with a wide dispersion of elemental ratios. Depletions are observed, however, in Ca, Ni and S relative to chondritic values. Shaded areas represent analyses of the matrices of 27 carbonaceous chondrites (3 C1, 11 C2 and 13 C3), and of matrix and chondrule rims from one unequilibrated ordinary chondrite (Tieschitz). The dispersion observed in element/Si ratios for Mg, Al and Fe in the matrices of carbonaceous and unequilibrated ordinary chondrites is of the same order as that observed for the unmelted particles. Similarly, the strong depletion of calcium is of the same order when the matrix is considered instead of the bulk. This depletion of Ca in the matrices of carbonaceous chondrites was recognized earlier by McSween and Richardson[10], who also found a depletion in S but no depletion in Ni.

The chemical composition of non-porous areas of fine-grained micrometeorites, as well as infrared absorption bands (1,580 and 3,260 cm^{-1}) of 12 similar unmelted particles[11], supports the existence of hydrous silicates and oxides in most of these particles. However, most Antarctic micrometeorites also contain anhydrous magnesium-rich olivines and pyroxenes. The coexistence of hydrous and anhydrous minerals indicates a similarity between micrometeorites and C2 carbonaceous chondrites. It also suggests some relationships with much smaller stratospheric IDPs. This view is supported by the frequency distributions of elemental ratios (Fig. 2), which are rather similar to those measured for hydrous IDPs[12].

Phase compositions, determined by electron microprobe analysis, independently support the extraterrestrial origin of the unmelted and partially melted scoria particles, as well as their relationship with carbonaceous chondrites. Phyllosilicate compositions are similar to those observed in C2 chondrites[10], with roughly chondritic abundances of Al, Ti, Cr and Mn, subchondritic abundances of Ni, Ca, Na and K, and highly variable FeO/MgO ratios (0.28-3.3 by weight). Olivines also have variable FeO/MgO ratios ($F_{O_{99}}$-$F_{O_{25}}$) with magnesian compositions predominating. Their minor element contents are usually high, with ranges of 0.1-1.0 wt% and 0.02-2.8 wt% for Cr_2O_3 and Al_2O_3, respectively, and most olivines have Fe/Mn ratios <C1 values (olivines enriched in Mn). These features are typical for olivines from carbonaceous chondrites[13-15] and IDPs[16] and are not compatible with either equilibrated ordinary chondrites or terrestrial olivines.

Pyroxenes are present in a variety of compositions. Low-calcium pyroxenes are mostly enstatites (iron-poor) and are rich in minor elements (Al, Cr and Mn). Of the calcium-rich pyroxenes, two varieties are present: diopside (rich in Al, Ti and Mn) and fassaite, a refractory pyroxene very rich in Al_2O_3 (16 wt%) and TiO_2 (0.7 wt%). All pyroxene compositions are typical for those found in carbonaceous chondrites and IDP pyroxenes[15,17]. The metal is nickel-rich (35 wt% Ni) and has a primitive Ni/Co ratio (~22) clearly indicating a primitive heritage (ordinary chondrites have fractionated metal compositions). Poorly characterized (probably hydrous) oxides are commonly nickel-bearing and have Ni/Co ratios similar to bulk C1 chondrites (~23). Low-nickel pentlandite (3.8-14.7 wt% Ni) is the main sulphide phase, also typical for carbonaceous chondrites and IDPs[17]. Some particles, like 89A-33 (Fig. 1*b*), which do not seem to have been melted, contain a glassy phase with a roughly chondritic composition which is depleted in nickel (and probably other siderophile elements). Such glasses are rare in C2 meteorites[18], but common in IDPs[19].

Noble gas analysis was performed using low-blank laser

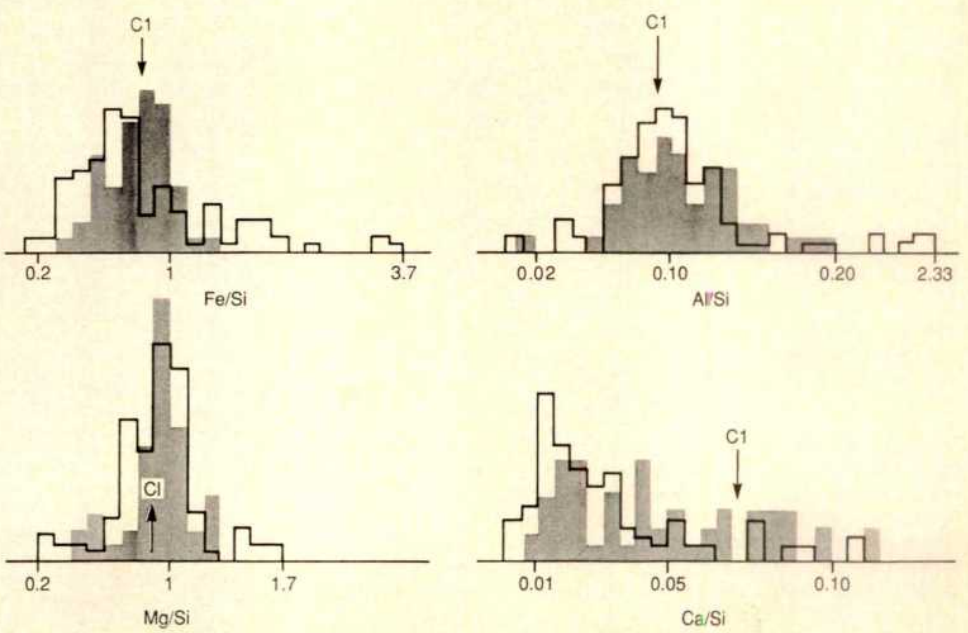

FIG. 2 Ratios of major element to silicon for Cap Prudhomme unmelted micrometeorites (full-line histograms) compared with matrices of carbonaceous chondrites and of the unequilibrated ordinary chondrite Tieschitz (shaded histograms) according to McSween and Richardson[10] and our own unpublished data. Multiple analyses were performed on micrometeorites and the carbonaceous chondrites at scales of ~10 μm (our data) and 100 μm (ref. 10) to assess heterogeneity and facilitate comparison with IDPs.

extraction techniques[20] on 19 unmelted and 28 partially melted chondritic particles considered 'probably extraterrestrial' as well as on 9 non-chondritic particles and 19 chondritic spherules. A total of 26 particles contained enough neon to permit isotopic measurements (for neon data, see Supplementary Information). Eighteen of these samples (9 unmelted, 7 partially melted scoria and 2 spherules) have isotopic compositions at least 2σ different from terrestrial neon, confirming their extraterrestrial origin (Fig. 3). (The others may also be extraterrestrial, but cannot be unequivocally confirmed as such.) Most of the gas-rich particles have neon isotopic compositions distinct from air, containing a mixture of neon from solar wind[21] and from solar energetic particles[22]. In addition, several particles have compositions displaced towards higher $^{21}Ne/^{22}Ne$ values, as would be expected for spallation neon, which is produced by cosmic-ray interactions with the silicates. The low, but measurable, spallation excesses observed in some grains can be attributed to irradiation by cosmic rays either when the micrometeorites existed as small particles in space or, possibly, during a previous residence time in the near-surface region of a larger parent body.

Particles analogous to Antarctic fine-grained unmelted particles are not generally observed in collections of less friable and more heavily weathered micrometeorites collected from Greenland[23] and deep-sea sediments[24]. In spite of these differences, isotopic results for all of these particles are nearly identical[6,25,26] and are similar to neon compositions measured in stratospheric IDPs[27]. This may reflect a similarity in their exposures to energetic particles in space.

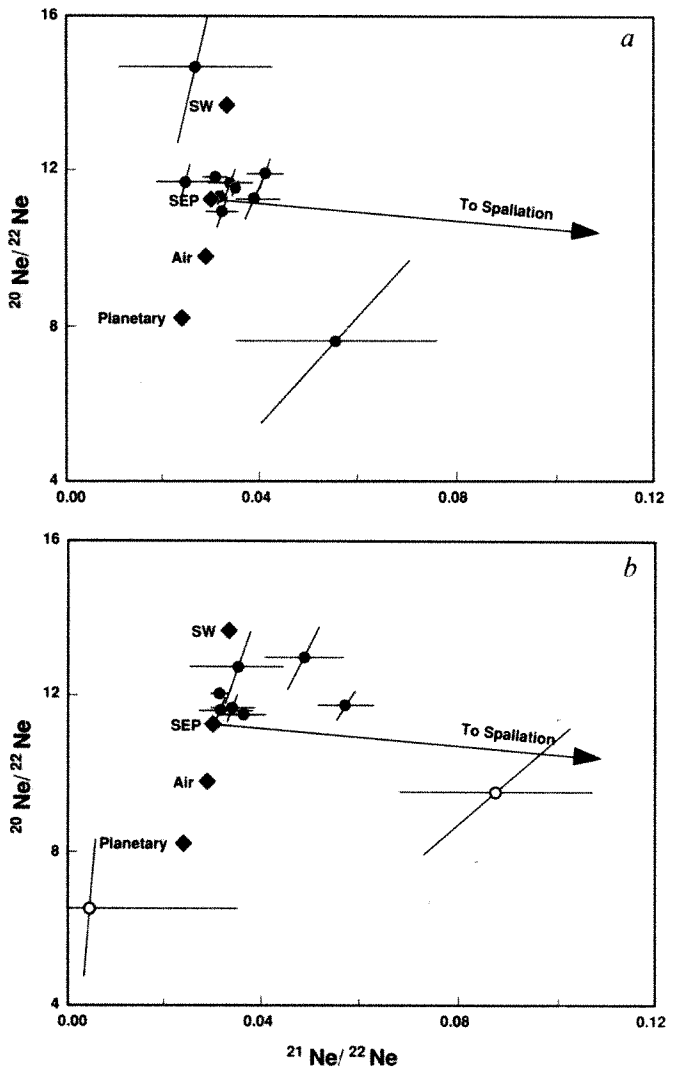

FIG. 3 Isotopic composition of neon extracted from Antarctic micrometeorites, with 1σ uncertainties. *a*, Unmelted particle (●). *b*, Partially melted scoria particles and melted spherules (open circles). Diamonds indicate the compositions of solar wind (SW), solar energetic particle (SEP), atmospheric (Air) and planetary neon. Most Antarctic particles with measurable neon are dominated by the surface-implanted solar components, demonstrating their extraterrestrial origin and suggesting that they are exposed in space as micrometeoroids.

Many of these particles have surprisingly high concentrations of implanted solar neon. Over half of the samples confirmed to be extraterrestrial by neon analysis have neon concentrations in excess of 10^{-5} cm^3 g^{-1} at STP. This constrasts sharply with larger meteorites, where all but five 'gas-rich' meteorites have neon concentrations below this value, and two of those are lunar meteorites[28]. These high concentrations, as well as the observation that most of the neon is implanted solar gas, strongly suggest that most of the samples were exposed in space as micrometeroids (they are not simply fragments of larger meteorites produced by ablation on entering the atmosphere).

Neon analysis confirms an extraterrestrial origin for only ~1/4 of the 'chondritic' particles studied, but these include representative samples of unmelted particles, partially melted scoria particle and, surprisingly, two of the melted cosmic spherules. Moreover, based on mineralogical, chemical and textural grounds (Fig. 1), gas-rich and gas-poor particles identified as 'probably extraterrestrial' are indistinguishable, except for a higher content of anhydrous mineral inclusions ≥ 5 μm in the gas-rich particles. It is therefore likely that most or all of the gas-poor chondritic particles are also micrometeorites.

We have given elsewhere[29] a rough estimate of the flux of micrometeorites (size range 50–300 μm) as recovered from either the Greenland or the Antarctica ice sheets, which agrees within a factor ≤ 3 with the total mass influx on the Earth (~20,000 tonne yr^{-1}). The Antarctic ice provides an inexhaustible source of micrometeorites that show an amazing diversity (no two grains are exactly alike in this set). Overall, in chemistry and mineral composition they have strong similarities with carbonaceous chondrites and IDPs. They are not, however, identical. Unmelted and partially melted scoria particles are depleted in both nickel and sulphur relative to carbonaceous chondrites and to IDPs[12]. One unmelted particle (89A-36) contains an olivine crystal with a NiO content (~0.4%) unknown in meteoritic olivines. If further work shows that such differences do not reflect weathering in the ice or frictional heating in the atmosphere, then they may represent a new population of Solar System objects. Further studies of these unique particles could broaden our knowledge of Solar System objects accreting to Earth today and may well provide new insights into nebular and pre-nebular processes. □

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Structural origin of reduced critical currents at $YBa_2Cu_3O_{7-\delta}$ grain boundaries

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THE critical current density across individual grain boundaries in thin films of the high-T_c superconductor $YBa_2Cu_3O_{7-\delta}$ (YBCO) has been found[1-4] to be inversely proportional to lattice misorientation for tilts up to ~10°. Reports of impurity segregation[5,6] at grain boundaries, and variations in the chemical stoichiometry[7,8], have led to the view that deviations from the ideal composition are responsible for the depressed superconducting order parameter at the boundary. Here we present images of YBCO grain boundaries obtained by a scanning transmission electron microscope in Z-contrast mode[9,10], which show that chemical segregation does not necessarily occur at these boundaries. A simple model of the strain associated with the grain-boundary dislocations provides a reasonable physical explanation of the suppressed superconductivity. The surprisingly large effect of strain implied by our model has implications beyond critical currents, for the physics and applications of any thin-film YBCO structures involving strained epitaxial layers.

In epitaxial thin films of $YBa_2Cu_3O_{7-\delta}$ on polycrystalline $SrTiO_3$, the critical current density across a grain boundary decreases drastically with increasing misorientation angle,[1-4] and the boundary behaves as a weak link for misorientations greater than 10°. Recent results from bulk-scale bicrystals, however, have indicated that certain high-angle grain boundaries do not exhibit this weak-link response[11]. No satisfactory microscopic explanation for the origin of this behaviour of grain boundaries has so far been proposed[3,12]. Additionally, it has structural models of low-angle boundaries do not lead to the observed angular dependence of the transport critical current[2,4,12]. The most commonly accepted view is that deviations from the ideal stoichiometry are responsible for the low critical current densities[13]. There are several reports of compositional variations at grain boundaries[5-8], but only small decreases in already low critical current densities have been directly correlated with deviations from the stoichiometric composition in the boundary regions[8].

We have used the Z-contrast technique[9,10] for forming chemically sensitive high-resolution images in a scanning transmission electron microscope (VG Microscopes HB501UX STEM operating at 100 kV) to investigate the composition of YBCO grain boundaries. In this technique, a fine electron beam of width 0.22 nm (full width at half maximum intensity) is scanned across the sample, and the electrons scattered at high angles are collected by an annular detector and used to form an image. The high-angle scattering is proportional to Z^2, where Z is the atomic number of the species under the probe. Thus the image can be thought of as a map showing at atomic resolution the scattering power of the sample[9,10]. This technique can provide analysis of regions of the sample down to a size comparable to the superconducting coherence length. We have examined boundaries of the same type as those investigated in refs 1-4. Such tilt boundaries consist of an array of uniformly spaced edge dislocations produced to accommodate the mismatch[14]. Our grain-boundary samples were taken from polycrystalline thin films of $YBa_2Cu_3O_{7-\delta}$ grown on Y_2O_3-stabilized ZrO_2 and from a pellet of sintered powder. Figure 1 shows Z-contrast images of a 13° tilt boundary at which the adjacent grains nearly share a common [100] direction. In Fig. 1*a* the beam passes parallel to the crystal planes of both grains, which are seen to be separated by an array of triangular defects. The planes of barium atoms ($Z = 56$) show higher intensity than the yttrium ($Z = 39$) planes, and terminate both grains along the long sides of the triangular zones. Phase-contrast imaging using the STEM bright-field detector clearly shows that these faceted dislocation cores are amorphous. To determine whether the amorphous material has the same composition as the crystalline superconductor, it is necessary to tilt the right-hand grain by a few degrees so that the clear image of the crystal planes is lost. The intensity scattered by the crystal in a random orientation will equal the intensity scattered by a random (amorphous) arrangement having the same overall composition. The contrast in the annular detector signal can be quantified very simply using appropriate screened cross-sections for elastic scattering[15]. Thus we can probe any segregation to the amorphous zones with a sensitivity limited by the image statistics and by the extent to which we can avoid thickness variations and residual channelling effects, which we estimate to be ±2%.

Within this limit no contrast changes are detectable between the amorphous zones and the randomly oriented crystal (Fig. 1*b*). Similar results were obtained from other tilt boundaries, including a 34° high-angle (high-energy) tilt boundary. This rules out the presence of any of the likely phases, including $Y_2Ba_4Cu_7O_{14}$, $YBa_2Cu_4O_8$ and $BaCuO_2$, and sets limits to the range of stoichiometry that would not be detectable as

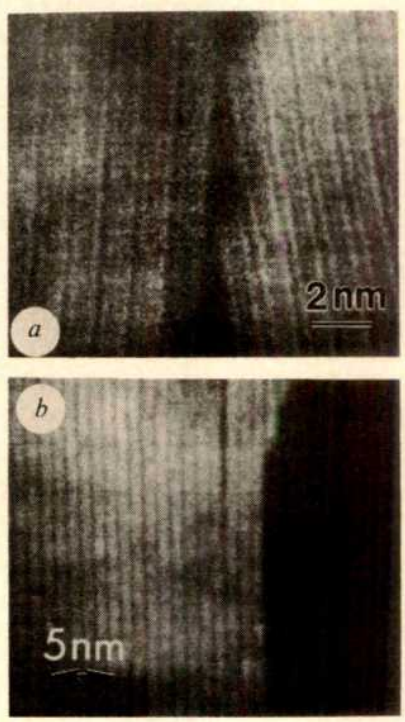

FIG. 1 Z-contrast images of a 13° [100] tilt boundary. *a*, Both grains oriented for electron channelling along the (001) planes, showing an array of triangular amorphous zones at the boundary. *b*, Sample tilted so that the right-hand grain is not channelling, showing no detectable contrast variations along the grain boundary.

$Y_{0.87\leq x\leq 1.13}Ba_{1.92\leq x\leq 2.07}Cu_{2.79\leq x\leq 3.21}O_{4.91\leq x\leq 9.09}$, where only one component is assumed to vary at a time. The method is rather insensitive to oxygen because of its relatively low atomic number, but given the high mobility of oxygen at low temperatures[16] it seems doubtful that any microscopic measurement on a thin specimen could provide meaningful oxygen contents. We estimate the spatial resolution of our measurement to be 1 nm, significantly better than has been achieved using X-ray fluorescence. This resolution is possible because the number of elastically scattered electrons detected is typically 10^5 times greater than the number of X-rays collected from the same area, allowing us to work with thicknesses of only 10–20 nm, which greatly reduces beam-broadening effects. Our results therefore represent the most sensitive measurements so far of grain-boundary composition. They indicate clearly that, although compositional variations undoubtedly can occur, they are not necessarily present.

In the light of these results, we therefore re-examine structural models of grain boundaries and attempt to understand how a dislocation array can cause a large reduction in critical current. We start with the simplest model and assume that the order parameter for superconductivity is depressed only within a strained region associated with the grain-boundary dislocations, which we assume has a well defined radius, r_m, in the plane of the boundary. Therefore, the ratio of the critical current density across a grain boundary, J_c^{gb}, to that within the grain, J_c^G, is $(D-2r_m)/D$, where $D=|\mathbf{b}|/\sin\theta$ is the dislocation spacing, $\mathbf{b}$ is the Burgers vector of the dislocation, and θ is the tilt angle of the two adjacent grains. For small tilt angles,

$$J_c^{gb}/J_c^G = 1-\frac{2r_m}{|\mathbf{b}|}\theta \qquad (1)$$

That is, the normalized grain-boundary critical current density decreases linearly with θ for small misorientations. In Fig. 2 we show a linear plot of the data of Dimos *et al.*[4], which clearly shows this predicted linear behaviour in the small-angle regime. From a least-squares fit of the slope between $\theta=0°$ and 10°, we determine $r_m\simeq 2.9|\mathbf{b}|$, which is much larger than the value assumed previously[2,6].

We believe that the physical origin for this surprisingly large effect of a grain-boundary dislocation is that the superconducting properties of $YBa_2Cu_3O_{7-\delta}$ are very sensitive to strain. Superconductivity is produced in this normally antiferromagnetic insulator by the addition of charge to the CuO_2 planes[17]. Disruption of the charge reservoir layers, for example by disordering the Cu–O chain layers, will affect superconductivity adversely. A strain of only 1% along the *a* or *b* axis is required to prevent the transformation from a tetragonal to an orthorhombic structure during processing, interfering with the order in the Cu–O chain layers and leading to significant volumes of non-superconducting material surrounding each dislocation core. Independent support for this 1% criterion comes from particle irradiation experiments in which oxygen is displaced with no overall change in oxygen stoichiometry[18,19]. Extrapolating the data to one oxygen displacement per unit cell, which would destroy the orthorhombicity, predicts T_c to be depressed by ~100 K. Further indications of the importance of strain comes from observations on $Nd_{1.83}Ce_{0.17}CuO_x/YBa_2Cu_3O_{7-\delta}$ multilayers, in which 1% strains significantly affected the normal-state resistivity and the superconducting transition[20]. We therefore adopt 1% strain as the criterion for defining r_m, and calculate the strain field around an edge dislocation array parallel to the tilt axis assuming that linear isotropic elasticity theory is applicable[21]. Figure 3 is a plot of the roughly elliptical area around the dislocations in which one of the strain components is calculated to be ≥1%. Reduced distances are used so that the solutions for 2°, 5° and 10° tilt boundaries can be displayed. It can be seen that for all three cases there is a 'conducting plate' of relatively undistorted crystal between dislocations. For the 10° boundary, however, the thickness of this conducting plate is less than the dimensions of the unit cell, and thus the structural order required for superconductivity is destroyed everywhere along the boundary. For misorientation angles less than 10°, the equations for the extent of the 1% strain predict, to first order, linear dependence on misorientation angle, consisent with the available experimental data. The strong coupling observed by Mannhart *et al.*[3] in bicrystal films with less than 10° misorientation, where the magnetic-field dependence of the grain boundary critical current density was similar to that of a single crystal, is entirely consistent with our observation that for tilts of up to 10° there are regions of the boundary that are largely unaffected by the dislocations. The grain-boundary dislocations effectively only reduce the area of the grain-boundary plane. Therefore, our microscopic characterization of the structure and composition of the boundaries, and our calculations of the dislocation strain field provide a consistent and physical explanation of the critical current behaviour of individual boundaries.

Beyond 10° misorientations, the boundary loses the required structural order for superconductivity, provided that it cannot relax to a lower-strain configuration, and becomes a weak link which is very sensitive to small applied magnetic fields[3]. Bulk-scale bicrystal results[11] and observations of special high-angle misorientations where a coincidence site lattice is produced[23] indicate, however, that there are grain boundaries that can undergo further relaxations to reduce the boundary energy; some of these may not behave as weak links. Unfortunately, the strain field at the boundary cannot be predicted solely by using geometrical factors that describe the boundary periodicity, but depends also on their specific atomic structure. The observation

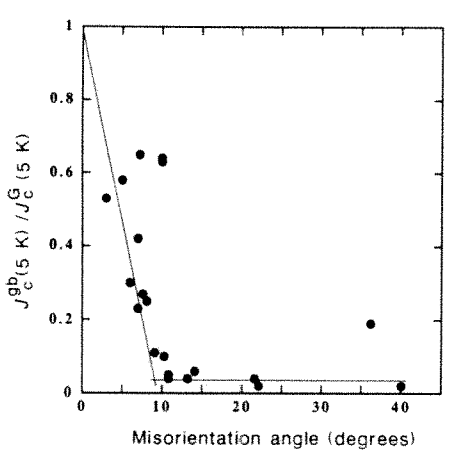

FIG. 2 Normalized critical current density as a function of misorientation[4], plotted on a linear scale.

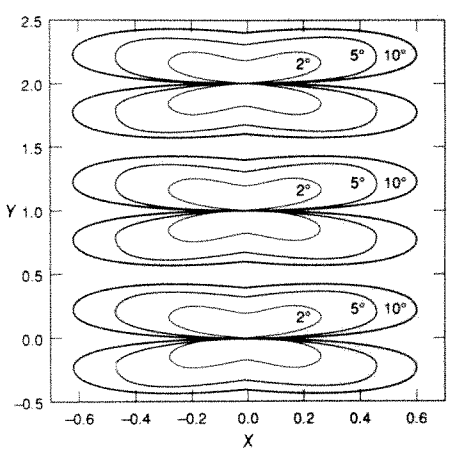

FIG. 3 Calculated strain field for an infinite array of edge dislocations at a 2°, 5° and 10° tilt boundary, showing the contour for a strain (ε_{xx}, the largest strain component) ≥1%, plotted using reduced distances $Y=y/D$ and $X=x/D$, where D is the dislocation spacing.

that a 14° boundary does not exhibit weak-link behaviour[11] does not necessarily contradict the strain model and, in fact, would seem to indicate that low-strain boundaries are not limited to high-coincidence orientations or, more probably, that the boundary contains segments of different structures, including a low-angle segment. The observation that 90° boundaries can sustain large critical currents[11,12] is entirely consistent with our model, because the dislocation array for such boundaries will be widely spaced. The structure of these and other special boundaries is currently under investigation. As it seems that the strain field, rather than the dislocations or boundaries themselves, is responsible for the suppression of superconductivity, the implications of our results are not limited to grain boundaries. The surprisingly large effect of strain will influence the superconducting properties of many artificial structures, such as superlattices and thin-film devices, which involve strains of the order of 1%. □

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Surface-mediated alignment of nematic liquid crystals with polarized laser light

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THE control of molecular alignment in liquid-crystal phases at macroscopic scales has been investigated extensively because of its importance in optical or optoelectronic applications, such as liquid-crystal displays[1]. It is well established that liquid crystals can be aligned by an applied electric field, a magnetic field, a shear-flow field, mechanical grooving of the substrate surface or stretching of liquid-crystal polymer thin films[2,3]. Here we report a new mechanism for liquid-crystal alignment that uses polarized laser light. We find that nematic liquid crystals in an illuminated region become oriented perpendicular to the direction of the electric-field polarization of the laser and remain aligned in the absence of the laser radiation. The liquid crystals can be reoriented again by subsequent illumination. This technique might have applications for large-area displays, optical memories, binary optics, adaptive optics and molecular micro-assembly.

We spin-coated a glass substrate with a thin layer of a silicone polyimide copolymer doped with a diazodiamine dye at a dye : polyimide weight ratio of 1 : 2. The chemical structure and absorption spectrum of the dye are shown in Fig. 1. We assembled a cell using this coated glass substrate as the top plate and a second glass substrate, coated only with polyimide, as the bottom plate, with a spacing of 11 μm between them. Both plates were rubbed with a cloth before assembly, the rubbing directions being matched on assembly. The cell was then filled with the nematic liquid crystal ZLI-1982 (EM Chemicals, Hawthorne, New York) at room temperature This is a eutectic mixture of phenylcyclohexane liquid crystal molecules. By using a polarization microscope, we observed the direction of the nematic phase to line up with the rubbing direction of the cell.

The cell was illuminated with a polarized argon ion laser (514.5 nm), with the direction of laser polarization parallel to the rubbing axis (Fig. 2). Within the illuminated region, the molecules of ZLI-1982 then assumed a twisted nematic structure. The molecules adjacent to the illuminated, dye-doped surface became oriented perpendicular both to their original direction and to the direction of the laser polarization, whereas molecules adjacent to the undoped polyimide surface remained aligned parallel to the rubbing direction. The image written in this way can therefore be seen with a pair of polarizers (Fig. 3). Similar control of liquid-crystal alignment was achieved by illuminating

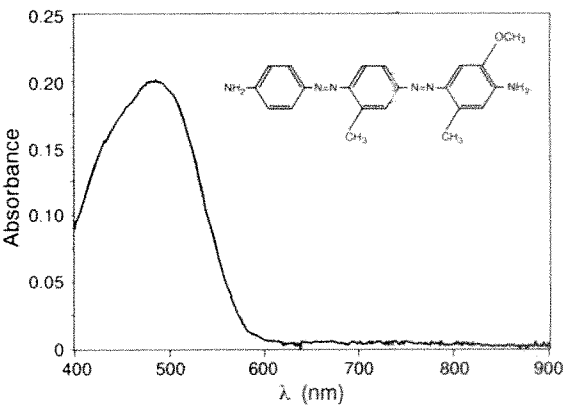

FIG. 1 The chemical structure and absorption spectrum of the dye dopant in the polyimide surface layer.

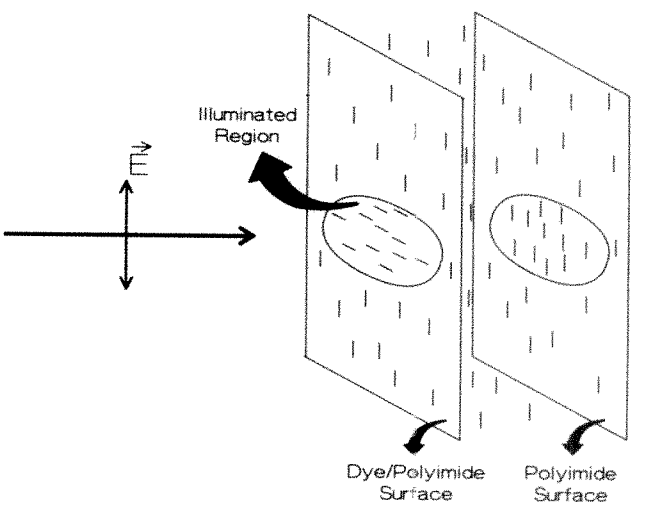

FIG. 2 The geometry of the illuminated liquid-crystal cell. The glass substrates of the cell are not shown for clarity. The rods represent the liquid-crystal orientation near the substrates before and after illumination.

the dye-doped polyimide substrate before filling with the liquid-crystal medium. A liquid-crystal 'grating' made in this way is illustrated in Fig. 4. The liquid-crystal alignment induced by laser irradiation is stable and can be erased or re-written by altering the direction of the incident electric-field polarization of the laser beam.

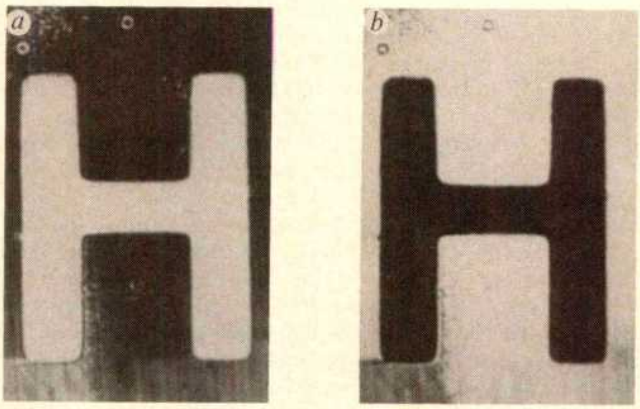

FIG. 3 A laser-aligned letter H in the liquid-crystal cell as viewed with a polarization microscope for *a*, parallel polarizers, and *b*, crossed polarizers. A mask in the shape of the letter H was placed in front of the liquid-crystal-filled cell, which was then illuminated with a 514.5-nm polarized laser at 9 W cm^{-2} for 1 min. The liquid-crystal alignment inside the letter H was along the initial alignment direction induced by rubbing the surfaces with a cloth. The liquid crystals within the illuminated background assumed a twisted nematic structure which caused a 90° rotation of the incident light polarization. The image has a height and width of 1.5 mm and 1.2 mm, respectively.

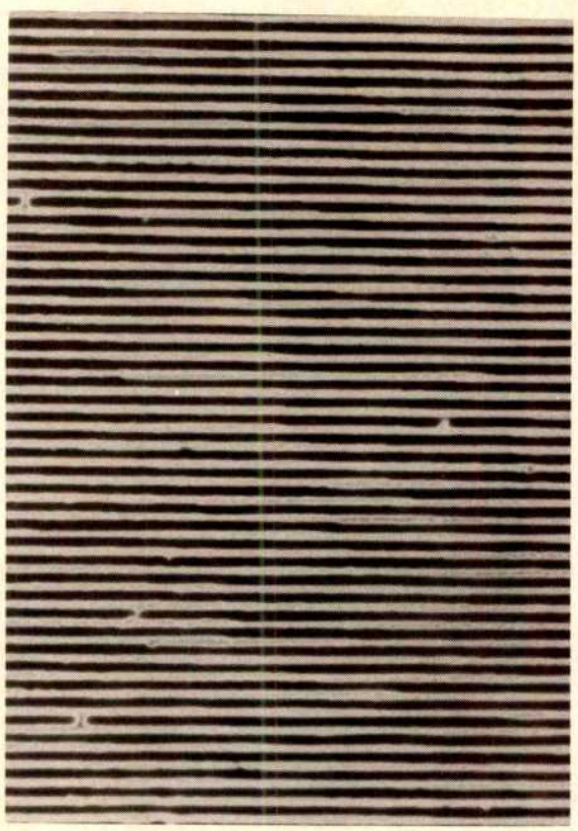

FIG. 4 A laser-aligned 10-μm grating in the liquid-crystal cell, as viewed with a polarization microscope with crossed polarizers. The cell was illuminated with a two-beam, plane-wave interference pattern with the angle between the plane waves chosen to give a fringe periodicity of 10 μm. The average incident intensity of the interfering beams onto the cell was 4 W cm^{-2}. The cell was exposed for 10 min. In this case, the cell was illuminated before filling with the liquid crystal.

An interesting aspect of our finding is that liquid crystals with a positive dielectric anisotropy are aligned perpendicular to the electric field of the laser beam. The 'memory' effect (alignment after irradiation of the dye-doped substrate) indicates that the laser-induced alignment is surface-mediated: the effect of irradiation of the dye-doped surface is apparently being 'read out' by the liquid crystal. Light-induced dye orientation and birefringence phenomena in a polymer matrix have been reported previously[4,5]. It is plausible that the orientation of liquid crystals directly reflects the state of dye orientation at the liquid-crystal/polymer interface. The mechanism of coupling between liquid-crystal molecule and dye-doped polymer is unclear at present. Our preliminary results have shown that the kinetics of laser-induced orientation is a function of dye structure, dye concentration, liquid-crystal type, incident energy density and polymer chemistry.

The possibility of controlling the orientation of liquid crystals at a spatial resolution of the order of micrometres or less suggests a number of potentially useful applications. Liquid crystals are birefringent, and switchable by either electric or magnetic fields. Diffractive optical elements can thus be made, such as phase gratings and binary holograms. Coupled with proper electrode design, optical devices useful for adaptive optics can be envisaged. Our experiments also demonstrated the use of the liquid crystal as a read-out device for the surface state. The ordering of the surface by the laser beam may have uses in molecular micro-assembly. □

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Insignificant isotropic component in the moment tensor of deep earthquakes

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THE mechanism responsible for deep-focus earthquakes, which occur at depths of 300–700 km in subducting slabs, has been a long-standing problem in geophysics. Unlike shallow earthquakes, deep earthquakes cannot be attributed to frictional instabilities across a fault plane, because of high frictional resistance to sliding at depth. A volumetric change associated with a phase transition, expected to occur at depth[1–3], is often invoked as the physical mechanism; if so, the resulting source mechanism should contain a major isotropic component. Although many researchers have attempted to observe such an isotropic component[4–10], no one has yet convincingly proved or disproved its presence. There exists a portion of the seismogram which is well suited to resolve the isotropic component of deep earthquakes but which has not been analysed by previous workers. Here I use this component in a systematic analysis of 19 large deep earthquakes, and show that no significant isotropic component (<10% of the seismic moment) exists. A sudden implosive phase change can thus be ruled out as the primary physical mechanism for deep earthquakes.

A point-source earthquake mechanism is most generally expressed by a symmetric second-order tensor called the seismic moment tensor[11,12] M_{ij} $(i, j = r, \theta, \phi)$. The moment-tensor formalism includes both the isotropic source model and a double couple (two perpendicular force dipoles, with equal magnitudes

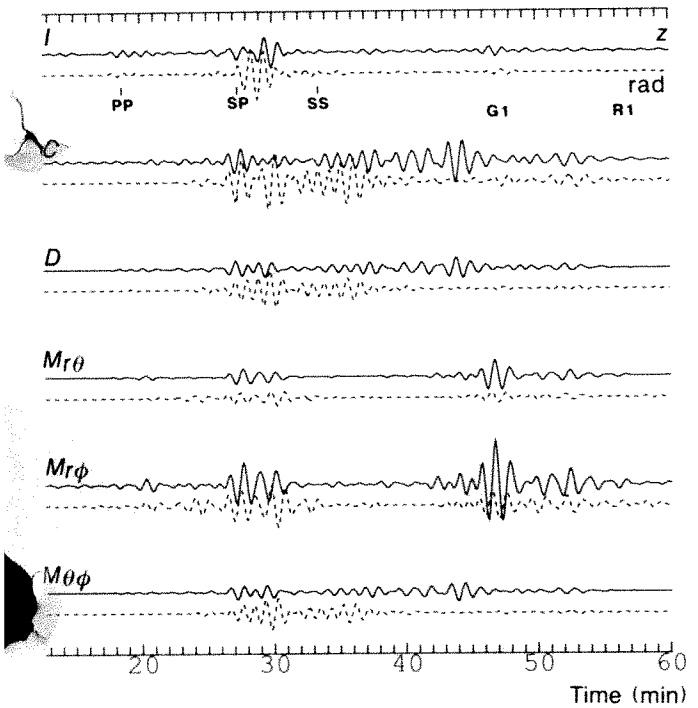

FIG. 1 Synthetic seismograms of long-period body waves for a deep earthquake. The earthquake is located at the hypocentre of the event south of Honshu and the station is BCAO (azimuth = 290°, Δ = 111°). The waveforms are calculated by a summation of normal modes and filtered between 10 and 22 mHz. For each pair of seismograms, the upper trace (solid line) is for the vertical component and the lower trace (dotted line) is for the radial component. Six pairs of seismograms are shown, one for each of six elements of the moment tensor. The diagonal components of a moment tensor are re-defined as $I=(M_{rr}+M_{\theta\theta}+M_{\phi\phi})/3$, $C=(M_{\theta\theta}+M_{\phi\phi}-2M_{rr})/3$, $D=(M_{\theta\theta}-M_{\phi\phi})/2$. Note that the body-wave waveforms for the isotropic component and C-component are quite different, but this is not the case for long-period surface waves. Expected arrival times of some important phases are also indicated.

but opposite signs), which corresponds to a slip on a planar fault, as special cases. A double-couple model has four degrees of freedom to specify a fault plane, a slip direction and a slip size, whereas a moment tensor generally has six degrees of freedom. These two excess degrees of freedom are called non-double-couple components of the moment tensor. It is common to separate a moment tensor into two parts, the isotropic component $I=(M_{rr}+M_{\theta\theta}+M_{\phi\phi})/3$ and the deviatoric part $M_{ij}-I\delta_{ij}$. Any deviatoric non-double-couple moment tensor can be expressed as a summation of two double couples, although the decomposition is not unique.

It is difficult to observe the isotropic component because the long-period surface-wave waveforms or narrow-band low-frequency normal-mode data excited by an isotropic source are very similar to those excited by another independent (deviatoric) component of a moment tensor, the vertical compensated linear vector dipole[4] (CLVD) component $C=(M_{\theta\theta}+M_{\phi\phi}-2M_{rr})/3$ (ref. 13). As most previous studies used only the above data, reported isotropic components, large or small, are subject to large uncertainty because of the presence of vertical CLVD components. Studies[4,14] using amplitudes of a few body-wave phases are also not promising, because many deep earthquakes are known to have complex source time-functions[15] and variable source mechanisms[16,17], and because the data coverage on the focal sphere is very limited. It is thus essential to analyse waveforms of many different body-wave phases to measure the isotropic component accurately.

The portion of the seismogram from the first P-wave arrival to just before the arrival of the first surface-wave train contains many different body-wave phases, including P, PcP, PP, PS and

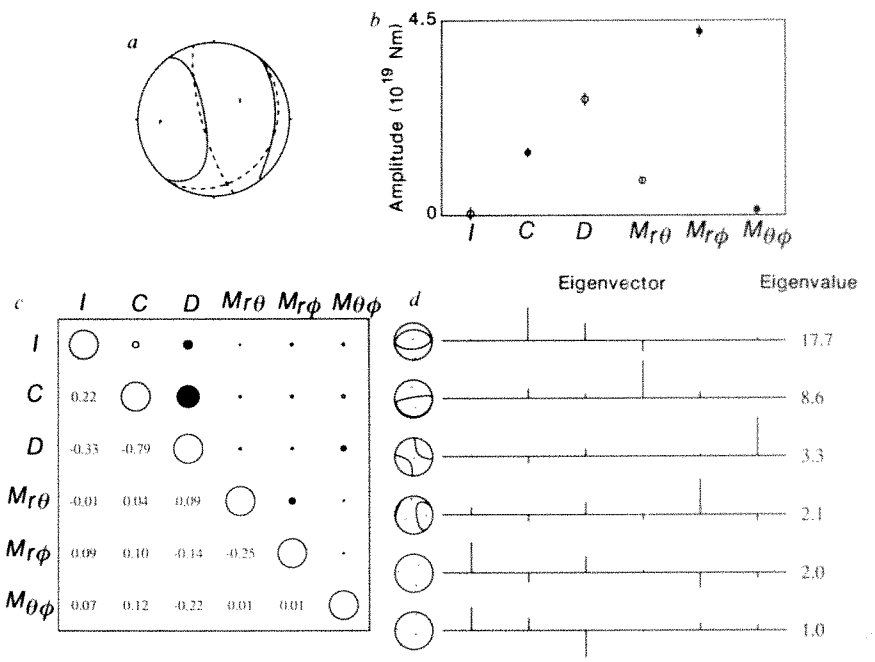

FIG. 2 Example of CMT inversion for the south of Honshu earthquake (1 January 1984, depth = 383 km). *a*, Equal-area projection (lower hemisphere) of the CMT solution. The solid lines show the nodal lines of the full moment tensor, with the shaded area indicating the positive first motion region. The dotted lines indicate the nodal lines of the double-couple model that shares the same principal axes as the non-double-couple (full) moment tensor. Note the large non-double-couple component. *b*, Relative amplitude of each component of the moment tensor. The error bars indicate one standard deviation. Open and closed circles indicate positive and negative values, respectively. Note that the isotropic component is almost zero. *c*, Corresponding correlation matrix. The diameter of circle is proportional to the magnitude of the correlation coefficient. Here the correlation coefficient between I and C is 0.22, whereas that with surface-wave data is 0.80, showing the superior resolution of I-component using long-period body-wave waveforms. *d*, Eigensolutions of the normal equation matrix. The lengths of the stick marks on each row give the contribution of moment-tensor elements for each eigenvector. The corresponding relative eigenvalues are given on the right and corresponding focal mechanisms on the left. A focal mechanism (that is, an eigenvector) that has a larger eigenvalue is better resolved with the given data set. The dots on the focal spheres indicate locations of the principal axes. Note that the eigenvectors that have significant isotropic components have the smallest eigenvalues.

others. Synthetic seismograms of such long-period (10–22 mHz) body waves for a deep earthquake indicate that the isotropic and the vertical CLVD components excite these waves quite differently (Fig. 1). It should therefore be possible to distinguish these two components by analysing long-period body waves; numerical experiments using these synthetic seismograms in fact prove that the isotropic component of a deep earthquake can be independently resolved by analysing long-period body-wave waveforms with sufficient station coverage (with stations every 30° both in longitudal and azimuthal directions, the correlation between two components can be <0.05 for deep earthquakes). Similar experiments with long-period (~150 to ~300 s) surface waves show a strong correlation between the isotropic component and the vertical CLVD component. The vertical CLVD component is often quite large for deep earthquakes. If the lateral heterogeneity of the Earth is not included in a model, such strong correlation with a presence of a vertical CLVD component can bias the estimate of the isotropic component[9,13].

The centroid-moment-tensor (CMT) inversion method of Dziewonski *et al.*[18], which simultaneously solves for a moment tensor and a spatio-temperoral centroid, is well suited for inverting these long-period body-wave phases to obtain the isotropic component of the moment tensor. Figure 2 shows the result of the CMT inversion of the above data for a deep (~380 km) earthquake which occurred south of Honshu, Japan on 1 January 1984. This event is well suited to test the method, because it has good station coverage and because it has a deviatoric non-double-couple moment tensor which is well documented[17,19] and large, indicating a somewhat unusual source mechanism. The correlation matrix indicates that the isotropic component is well resolved; the isotropic component obtained is essentially zero. Figure 2*d* indicates that the eigenvectors that have singificant isotropic components have the smallest eigenvalues. This suggests that it is still relatively difficult to observe the isotropic component even using long-period body-wave data. This is because the isotropic component is less efficient in exciting seismic waves in general, but the solution at least will not be biased by the presence of the vertical CLVD component.

To ascertain whether or not this result is general, we performed systematic body-wave CMT inversions for two sets of deep earthquakes. The first set consists of 9 events (including the earthquake on 1 January 1984) whose reported moment-tensor solutions[20] exhibit large deviatoric non-double-couple components. The second set consists of 10 events whose seismic moments are greater than 10^{19} N m. Figure 3 shows the moment-tensor solutions of the events in the first set. For all events that have large deviatoric non-double-couple components, the isotropic components are zero or negligible. Thus the large non-double-couple moment tensor observed for those events is entirely of deviatoric origin. For the event of 1 January 1984 it has been shown[17] that the large non-double-couple component observed at low frequencies can be explained by the presence of multiple subevents with different double-couple focal mechanisms separated in time by a few seconds, and does not require any isotropic component.

Figure 4 summarizes the size distribution of the isotropic component for all 19 deep earthquakes studied. The isotropic component is always ≤10% of the deviatoric seismic moment. Error bars covering two standard deviations always cut through the zero isotropic value. There seems to be no systematic difference between events with large non-double-couple components and those without. The sign of the isotropic component shows no systematic pattern, whereas an implosive phase change should always result in a negative isotropic component. These

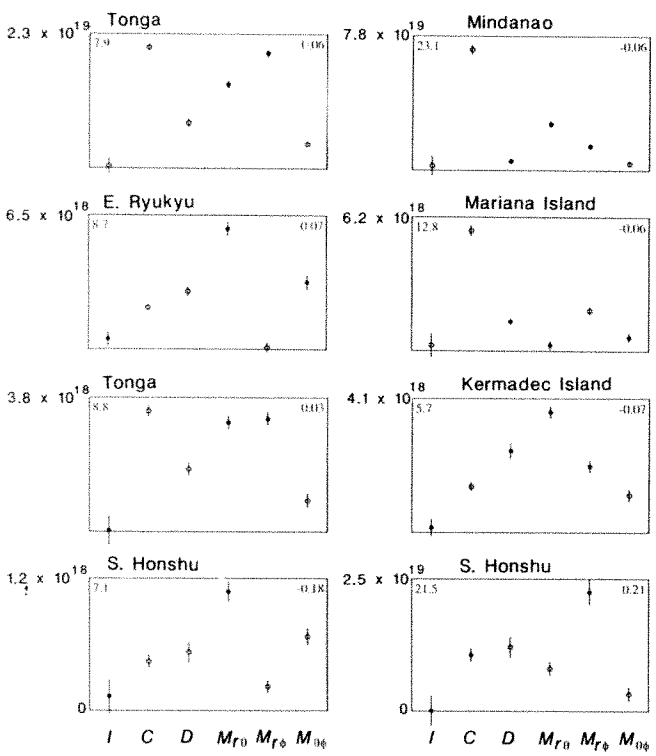

FIG. 3 Moment-tensor solutions for eight deep earthquakes which have a large deviatoric non-double-couple component (symbols as for Fig. 2*b*). The dates and depths of these earthquakes are: Tonga, 16 June 1986, 565 km; Mindanao, 5 March 1984, 630 km; east of Ryukyu, 4 July 1982, 554 km; Mariana Island, 4 January 1982, 590 km; Tonga 7 October 1981, 627 km; Kermadec Island, 28 September 1981, 335 km; south of Honshu, 31 March 1980, 359 km; south of Honshu, 7 March 1978, 434 km. The correlation coefficient between the *I* and *C* components, and the ratio of the maximum to minimum eigenvalues are shown at the right-upper and left-upper corner of each box, respectively. The ratio gives the condition number of the normal equation matrix; the smaller the ratio, the more stable the solution. Note that the vertical CLVD component (*C*) is often quite large for these events, and this can bias estimates of the isotropic component using long-period surface wave or free oscillation data. For all events, the isotropic components are essentially zero or negligible.

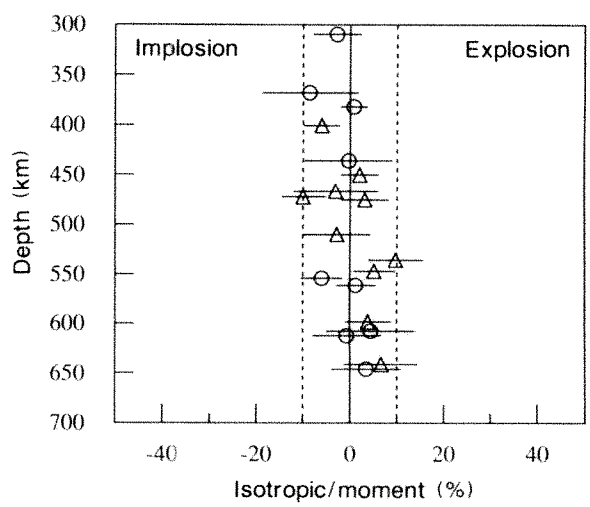

FIG. 4 Depth distribution of the isotropic components of all 19 deep earthquakes. The horizontal axis shows the size of the isotropic component as a percentage of the deviatoric seismic moment. The positive and negative values correspond to explosive and implosive isotropic sources, respectively. A deviatoric seismic moment is the average of the absolute values of the largest and smallest eigenvalues of a deviatoric moment tensor, $D_{ij} = M_{ij} - I \cdot \delta_{ij}$. The circles are for the nine events with large deviatoric non-double-couple components, and the triangles are for the ten events with seismic moments $>10^{19}$ Nm. The horizontal error bars indicate one standard deviation. The vertical broken lines indicate ±10% isotropic values and all points are within this range. There seems to be no systematic pattern and no difference between the two sets of deep earthquakes.

observations also hold for smaller deep events[21]. I therefore conclude that the isotropic component of deep earthquakes is <10% of the seismic moment in the period range 45–100 s, that its presence is statistically insignificant, and that a sudden implosive phase change can be ruled out as the primary physical mechanism for deep earthquakes. This conclusion is consistent with recent laboratory experiments on metastable phase transformations[22,23], which suggest 'transformation faulting' as the physical mechanism of deep earthquakes without requiring a significant isotropic component[1]. □

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Lower-mantle viscosity constrained by seismicity around deglaciated regions

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KNOWLEDGE of the viscosity structure of the Earth's mantle is important for constraining models of mantle convection and isostatic rebound. Here we show that seismicity around the margins of deglaciated areas provides a constraint on the viscosity of the lower mantle, in addition to those previously proposed[1,2]. Calculations using a spherical, viscoelastic Earth model show that the present-day magnitude of the stress fields induced in the lithosphere beneath the (now-disappeared) Laurentide and Fennoscandian ice sheets is very sensitive to the value of the lower-mantle viscosity. Stresses of ~100 bar, sufficient to cause seismicity, can still remain in the lithosphere for lower-mantle viscosities greater than $\sim 10^{22}$ Pa s; for lower-mantle viscosities of $\sim 10^{21}$ Pa s, only a few tens of bars of stress persist in the lithosphere today. This influence of lower-mantle viscosity on the state of stress in the lithosphere also has implications for the migration of stress from earthquakes, and hence for earthquake recurrence times.

Estimates of mantle viscosity have been based on postglacial rebound[1] and geoid anomalies[2]. A stratified mantle with a lower-mantle viscosity of $\sim 10^{22}$ Pa s is favoured by geoid anomalies[2], whereas from global sea-level changes[3] a lower-mantle viscosity has been obtained that is slightly higher than the upper-mantle viscosity of 10^{21} Pa s. Lower estimates for the upper-mantle viscosity of $3\text{–}5 \times 10^{20}$ Pa s can be derived from sea-level curves in northwestern Europe[4]. The issue of mantle viscosity structure is by no means settled because of the intrinsic differences in the various data sets.

The seismic activity at passive continental margins in eastern Canada and Fennoscandia has been attributed to the melting of the Pleistocene ice sheets[5–7]. Estimates of stress fields[8] produced by removal of ice loads were based on the elastic plate flexure model and showed that considerable stresses ($\sim 10^2$ bar) could be produced on unloading. Stresses from glacial melting were also computed using a viscous rheology in a spherical model with both uniform and stratified viscosity profiles. For a uniform mantle viscosity[9], stresses of a few bars were found at the base of the lithosphere, whereas for individual long-wavelength harmonics[10] in the stratified models, much higher stresses ($\sim 10^2$ bar) still remained at the top of the mantle, and the lower-mantle viscosity was $\sim 10^{22}$ Pa s. For the timescales considered here ($\sim 10^4$ yr), mantle deformation processes are better described by viscoelastic rheology. A more realistic model is needed to investigate the problem of contemporary stress fields induced by deglaciation; this model should incorporate viscoelastic relaxation, as the consequences of stress fields in former deglaciated regions can affect the assessment of earthquake hazards[11].

We have employed a five-layer model[12], consisting of a purely elastic lithosphere, a three-layer viscoelastic mantle with a Maxwell rheology and an inviscid core, to provide a realistic description of the evolution of stress caused by deglaciation and to account for the presence of a high-viscosity transition zone[13] in the mantle. The shear modulus and density for each of the layers is taken from seismic estimates. The ratio of lower- to upper-mantle viscosity is denoted by B; in models with a high-viscosity transition layer, there is another parameter C, representing the ratio of the transition-zone viscosity to the upper-mantle viscosity.

Green's functions can be constructed analytically for the stress fields from the elastic and viscoelastic contributions to the displacement fields. For the axisymmetric spherical model, the viscoelastic stress-tensor components in the Laplace-transformed domain are:

$$\sigma_{rr}(r, \theta; s) = \Pi + 2\mu(s) \sum_{n}^{K} \dot{U}_n P_n \tag{1}$$

$$\sigma_{r\theta}(r, \theta; s) = \mu(s) \sum_{n}^{K} \left[\left(-\frac{V_n}{r} + \dot{V}_n \right) P_n - \frac{U_n}{r} \partial_\theta P_n \right] \tag{2}$$

$$\sigma_{\theta\theta}(r, \theta; s) = \sigma_{rr} + 2\mu(s) \sum_{n}^{K} \left[\left(\frac{U_n}{r} - \dot{U}_n - \frac{n(n+1)V_n}{r} \right) P_n - \frac{\cot\theta}{r} V_n \partial_\theta P_n \right] \tag{3}$$

$$\sigma_{\phi\phi}(r, \theta; s) = 2\sigma_{rr} - \sigma_{\theta\theta} + 4\mu(s) \times \sum_{n}^{K} \left(\frac{U_n}{r} - \dot{U}_n - \frac{n(n+1)V_n}{2r} \right) P_n \tag{4}$$

where r and θ are respectively the radius and the co-latitude coordinate, ϕ is the longitude, s is the Laplace-transformed variable, n is the angular order of the Legendre function $P_n(\cos\theta)$, σ_{ij} are the elements of the stress tensor in an axisymmetric spherical model, Π denotes the isotropic components, and the scalar functions U and V represent respectively the radial and tangential displacements. The dot denotes differentiation with respect to r. The index K, here taken to be 100, represents the highest angular order used in summing the contributions from different wavelengths excited by the disintegrating ice sheet. These stress fields are time-dependent because the transformed shear modulus $\mu(s)$, U_n and V_n are all functions of the Laplace-transformed variable s. A linear Maxwell

rheology is used in $\mu(s)$ with an intrinsic shear modulus μ_0 and long-term viscosity ν_0 for each of the layers[14]. This rheology has been commonly used[2,12] in studying post-glacial rebound and changes from elastic to viscous behaviour over intermediate times of $\sim 10^3$ yr.

We have determined the evolution of the stress fields after a sequence of 10 glacial cycles with a period of 10^5 yr. The history of the displacement and stress fields is calculated for the past 12,000 years. We have investigated the stress fields induced by both the Laurentide and Fennoscandian ice sheets: these are modelled as circular disks that had fixed angular radii of 15° and 8°, respectively, and a parabolic profile along a vertical cross-section with initial maximum heights of 3.5 and 2.5 km respectively. The shrinkage of the ice sheets is controlled by decreasing the central peaks linearly with time. The details and the spectral coefficients used for the load are given in ref. 14.

Stress fields induced by deglaciation are influenced by stratification of the viscosity, as shown in Fig. 1 for a sequence of vertical sections of the top 400 km of the mantle. Each panel portrays a half cross-section passing through the axis of symmetry of the model, which is at the left. In Fig. 1*a*, we show a sequence of snapshots of $\sigma_M - \sigma_m$, the difference between the maximum and minimum eigenvalues of the stress tensor, for viscosity contrasts $B = 1$, 5, 10, 50 and 100. Each of the columns of the four quadrants denotes an individual physical model. The ice load is representative of the Laurentide ice sheet with an elastic lithosphere of 100 km thickness.

The stress fields at $t = 0$ (12,000 years ago) represented the cumulative effects from previous ice ages. There was a migration of stress from the mantle and a subsequent concentration of stress in the lithosphere (indicated by the red colour) at the edge of the ice sheet (0.4 the width of the panel), which spread downward into the mantle (green colour). After 6,000 years there was a build-up of stress in the lithosphere beneath the load for lower-mantle viscosity $\geq 5 \times 10^{21}$ Pa s or $B \geq 5$. Today, the lithospheric stress fields have decreased somewhat, as shown by the disappearance of the green colour at the edge of the former ice load. For a lower-mantle viscosity of 10^{21} Pa s, a value commonly inferred[1] from post-glacial uplift, the stresses induced by deglaciation in the lithosphere are very small (blue colour), around 20 bar. Thus the amount of residual stress from the last ice age increases markedly for lower-mantle viscosity between 10^{21} and 10^{22} Pa s and flattens out if the mantle is highly stratified ($B > 50$). The thickness of the lithosphere can also influence the stress distribution: Fig. 1*b* shows the results for a lithosphere of thickness 150 km, characteristic of continental cratons[15] beneath the Canadian ice sheet. A thicker lithosphere supports less residual stress, as can be seen by the lighter hues of red at $t = 12{,}000$ yr. For a lower-mantle viscosity of 10^{22} Pa s, a decrease in the stress levels of ~20% is found today. The trend of increasing stress with increasing lower-mantle viscosity is similar to that for the thinner lithosphere.

Stress fields are also affected by the lateral extent of the ice load. In Fig. 1*c*, we examine the effects of a smaller ice load such as that of the Fennoscandian ice sheet (model described above). Initially larger stresses, as shown by the green colour, are built up in the mantle and lithosphere (red colour) because the smaller size of the Fennoscandian ice sheet tends to focus the stress. Thereafter the stresses increase and then decrease to present-day values, which are comparable with those associated with the Laurentide melting for the same lithospheric thickness of 100 km (compare with Fig. 1*a*). Inspection of the bottom row shows that the effects of the lower-mantle viscosity on lithospheric stress fields are still evident today and are not influenced by the smaller size of the load.

Recent laboratory investigations[13] and Monte Carlo inversion of geophysical surface signatures[16] have indicated that the transition zone of the mantle at a depth of 400–670 km may be different from both the upper and lower mantles. Figure 1*d* shows the results of such a high-viscosity layer on the stress fields produced by melting of the Laurentide ice sheet over a lithosphere 150 km thick. We compare the case with $B = 10$ and high viscosity, $C = 50$ (panel A), with the cases where $B = 1$ and $C = 10$, 50 and 100 (panels B, C and D). The dominant influence of the lower-mantle viscosity over the high-viscosity layer is shown by the large stresses (yellow–orange) remaining in the lithosphere.

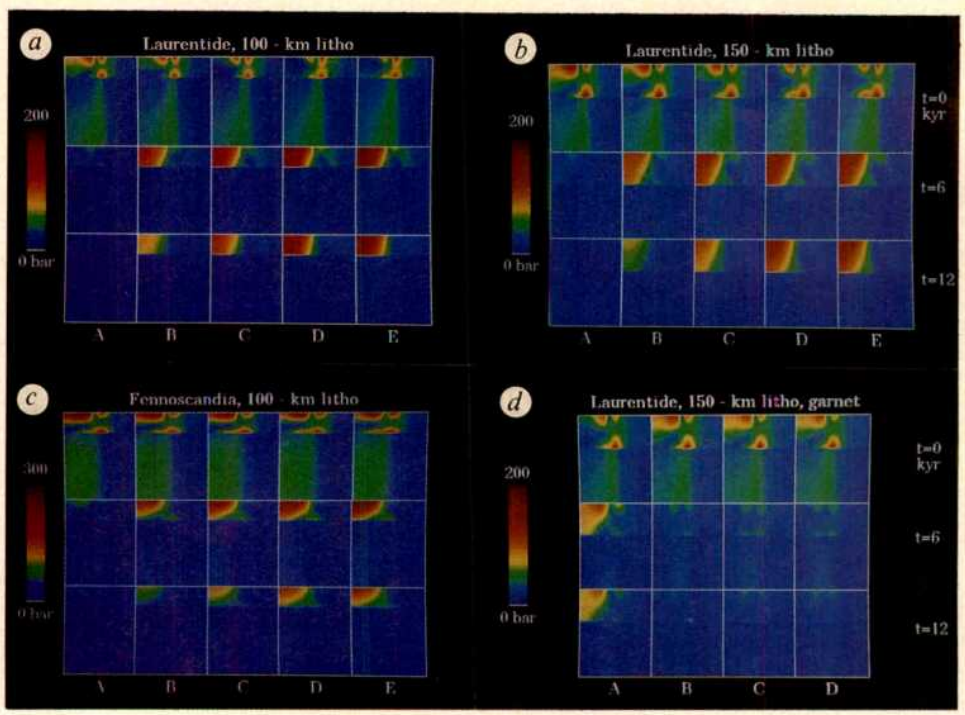

FIG. 1 *a*, Evolution of the $\sigma_M - \sigma_m$ field for the Laurentide ice sheet. σ_M and σ_m are the maximum and minimum principal stress values, respectively. The lithosphere thickness is 100 km. The panels depicting $t = 0$ do not include the elastic contributions from the instantaneous melting but reflect the contributions from the previous ten ice-age cycles with a period of 10^5 yr. Model A portrays an isoviscous mantle with a viscosity of 10^{21} Pa s ($B = 1$). Columns B, C, D and E have lower-mantle viscosities of 5×10^{21}, 1×10^{22}, 5×10^{22} and 10^{23} Pa s respectively ($B = 5$, 10, 50 and 100). The depth of each panel is 400 km, and the width is 2.5 times the angular radius of the ice-sheet, here taken to be 15°. The colour palette has 25 evenly spaced intervals and is linear. Maximum value of $\sigma_M - \sigma_m$ is <200 bar (deep red). *b*, As in *a*, with a 150-km-thick lithosphere. *c*, Evolution of $\sigma_M - \sigma_m$ field for Fennoscandian ice sheet with a 100-km-thick lithosphere. The angular radius of the ice sheet is 8°. Same viscosity models ($B = 1$, 5, 10, 50 and 100) as in *a*. Scales of the colour palette go up to 300 bar. *d*, Evolution of $\sigma_M - \sigma_m$ for Laurentide ice load with a high-viscosity transition zone. The lithosphere thickness is 150 km. Model A has an upper-mantle viscosity of 10^{21} Pa s, a transition-zone viscosity of 5×10^{22} Pa s ($C = 50$) and a lower-mantle viscosity of 10^{22} Pa s ($B = 10$). Models B, C and D all have upper- and lower-mantle viscosities of 10^{21} Pa s and transition-zone viscosities of 10^{22}, 5×10^{22} and 10^{23} Pa s ($C = 10$, 50 and 100). Maximum $\sigma_M - \sigma_m$ (deep red) is 200 bar.

On the other hand, even for an extremely-high-viscosity transition zone ($C = 100$, panel D) the additional stress produced in the lithosphere is small, as shown by the faint blue colour.

We have quantified the stress induced in the lithosphere by calculating the volumetric average $\bar{\sigma}$ of $\sigma_M - \sigma_m$ over the thickness of the lithosphere and out to a horizontal distance of 1.5 times the radius of the ice sheet. The temporal evolution of $\bar{\sigma}(t)$ for the various models presented in Fig. 1 is shown in Fig. 2. The lower-mantle viscosity exerts the greatest influence on the evolution of the lithospheric stress. We observe a non-monotonic behaviour of $\bar{\sigma}$ as a function of time for lower-mantle viscosities $\geq 2 \times 10^{21}$ Pa s. This is due to the delayed relaxation of stress in the lower mantle, which was not considered previously. For these viscosities, lithospheric stresses decay with characteristic timescales much longer than those associated with a lower-mantle viscosity of $\sim 10^{21}$ Pa s. This has important implications for questions of seismic risk in regions that lay beneath large ice sheets in the late Pleistocene, such as eastern Canada and Fennoscandia.

The quantity $f = \sigma_{\theta\theta} - \sigma_{rr}$ yields important information about the stress state of the lithosphere. Negative and positive values of f represent tensional and compressional states, respectively. The stress pattern of f changes when the lower-mantle viscosity is increased to 5×10^{21} Pa s. It is characterized by tension in the lithosphere below the former ice sheets and by compression beneath the surrounding unglaciated region. This pattern agrees with seismic focal mechanisms[7].

We have demonstrated that the stress fields induced by deglaciation 12,000 years ago are strongly influenced by the lower-mantle viscosity. For a highly viscous lower mantle (viscosity $\geq 10^{22}$ Pa s), an ice load characteristic of the late Pleistocene will give lithospheric stresses of 100–200 bar. These stresses could be large enough to induce seismic activity on faults that originated during previous tectonic episodes. Earthquake activity along passive margins of eastern Canada and Fennoscandia is more consistent with a highly stratified viscous mantle.

Spatial and temporal changes of the stress fields in the mantle are also induced by abrupt motions along faults in earthquakes. Conventional viscoelastic models[17,18] for the stress migration from earthquakes are based on the assumption of lithosphere–asthenosphere coupling[18,19] and do not consider the effects of stratification of mantle viscosity at deeper levels. From our time-dependent calculations for stress redistribution after deglaciation, we propose that for large earthquakes, stress transfer by the stress-diffusion mechanism could have a long timescale ($\sim 10^4$ yr), because of increased viscosity in the lower mantle. □

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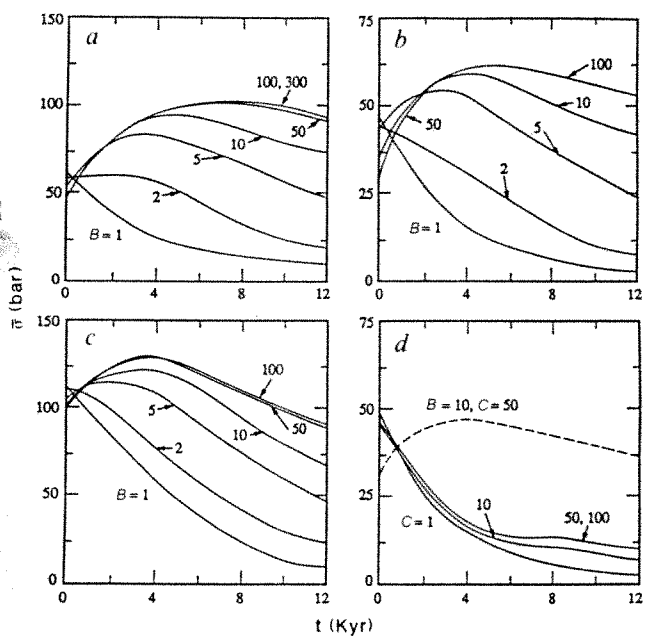

FIG. 2 History of volume-averaged $\sigma_M - \sigma_m$ for the various models. The average is taken over the thickness of the lithosphere and a horizontal distance 1.5 times the radius of the ice sheet. Panels *a*, *b*, *c* and *d* summarize the evolution of $\sigma_M - \sigma_m$ in Fig. 1. *a* and *c* are for a lithosphere thickness of 100 km, *b* and *d* for a thickness of 150 km. The dashed curve in *d* represents a model with both a high-viscosity transition zone ($C = 50$) and a high-viscosity lower mantle ($B = 10$). *B* and *C* are, respectively, the ratios of lower- to upper-mantle viscosity and of transition-zone viscosity to upper-mantle viscosity.

Low iron requirement for growth in oceanic phytoplankton

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DESPITE the controversy on the importance of iron in limiting phytoplankton growth and affecting air–sea exchange of CO_2 in the ocean[1–4], there is very little information on cellular iron requirements for growth. The few data available[5,6] come from species isolated from coastal sea water where dissolved Fe levels are 10–1,000 times higher than those (≤0.1 nM) in the open ocean[1,7]. Species from oceanic waters require much lower external Fe concentrations for growth than do comparable coastal species[8]. Here we report that an oceanic diatom was able to grow at a near maximum specific rate of about 1.0 per day at a cellular Fe:C ratio of 2 μmol:mol, about 25% of the amount needed for the same rate in a related estuarine species, and 2–20% of values previously used to estimate algal Fe requirements in sea water[1,2]. These results have important implications concerning iron limitation of primary productivity in the ocean and cell biology of iron in oceanic algae.

We measured the effect of Fe concentration on cellular Fe:C ratio and growth rate in *Thalassiosira oceanica* (clone 13-1), isolated from the Sargasso Sea, and *T. pseudonana* (clone 3H)

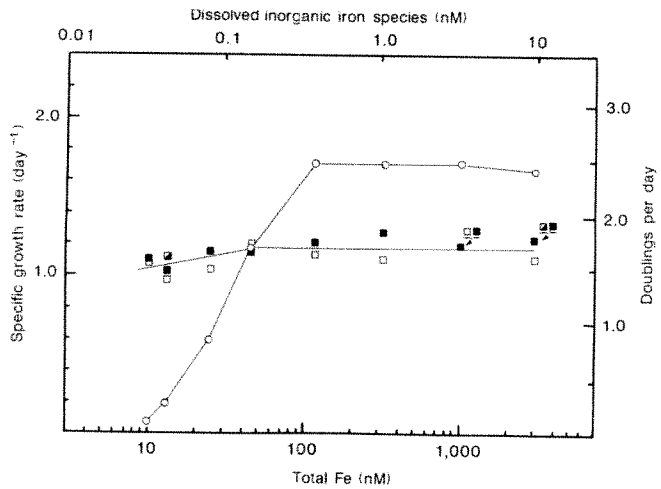

FIG. 1 Growth rate of clones 3H (○) and 13-1 (three successive experiments (◪, ■, □)) as a function of total Fe and mean concentration of dissolved inorganic Fe species. Cells were preacclimated at low total Fe (10 nM for 13-1, 45 nM for 3H) for 7–19 days and grown at 20 °C and pH 8.10 ± 0.06 under 14:10 h light:dark cycle (600 μmol quanta $m^{-2} s^{-1}$) in filtered (0.4 μm) Gulf Stream sea water (stored in the dark at 7 °C for 1 year). Sea water contained added nutrients (35 μM $NaNO_3$, 1.5 μM Na_2HPO_4, 40 μM Na_2SiO_3, 0.1 μg l^{-1} vitamin B_{12}) and a trace metal ion buffer system (0.1 mM EDTA, 100 nM $CuCl_2$, 250 nM $ZnCl_2$, 120 nM $MnCl_2$, 100 nM $CoCl_2$). Total Fe was computed from the sum of the background concentration (10 nM as measured by atomic absorption spectrophotometry following organic extraction[18,19]) plus Fe added with radiotracer and as unlabelled $FeCl_3$. Log free-ion concentrations of Cu^{2+}, Zn^{2+}, Co^{2+} and Mn^{2+}, were computed from equilibrium theory[11,20] and are −13.12, −10.42, −10.63, and −8.14, respectively. The mean dissolved inorganic Fe concentration [Fe′] was computed from conditional formation, dissociation and photodissociation rate constants for iron-EDTA in sea water[6]. Computed [Fe′]/[total Fe] = 0.00077 in dark and 0.0053 in light giving weighted mean of 0.0034 for 14:10-h light:dark cycle.

from a eutrophic estuary[9]. Experimental procedures and conditions were similar to those in previous culture studies with ^{54}Mn (refs 10, 11). Algae were grown in filtered Gulf Stream sea water containing added nutrients, $FeCl_3$ radiolabelled with ^{55}Fe and an EDTA-trace metal ion buffer system that reduced available inorganic Fe to levels far below total Fe concentrations. Growth rate was measured from daily increases in total cell volume using a Coulter counter. Intracellular Fe was determined in exponentially growing cells by filtering them onto 3-μm-pore Nucleopore filters, rinsing briefly with a Ti(III)EDTA-citrate reducing solution[12] to dissolve iron hydroxides and desorb ferric ions bound to cell surfaces, and measuring the remaining particulate ^{55}Fe by liquid scintillation counting. In some instances the cells were rinsed with sea water instead of reducing solution to measure total (intracellular plus surface) cell Fe. Cell ^{55}Fe values were corrected with filter blanks using media without cells. The fraction of ^{55}Fe in the cells was multiplied by the total Fe concentration and divided by the measured volume of cells per litre to yield cell Fe concentrations. These were converted to Fe:C ratios using cell carbon:volume ratios of 22 and 15 mol C l^{-1} for clones 3H and 13-1 that we determined with standard ^{14}C techniques and Coulter counter measurements of cell volume. Carbon:volume ratios were unaffected by Fe concentration (data not shown).

Decreases in Fe concentration reduced growth rate more in the neritic clone 3H than in the oceanic clone 13-1 (Fig. 1) in accordance with previous results[8]. Clone 3H grew 50% faster than clone 13-1 at high Fe concentrations; but at the lowest Fe level, its growth rate decreased to near zero while that of clone 13-1 was reduced by only about 15%. The ability of clone 13-1 to grow faster than clone 3H at low Fe levels was due almost entirely to a much lower cellular iron requirement for growth (Fig. 2), and not to a greater ability to accumulate iron (Fig. 3*a*). When intracellular Fe:C ratios were multiplied by specific growth rates, the resultant specific cellular Fe uptake rates were very similar for the two species at low growth-limiting Fe levels (Fig. 3*b*).

Oceanic algae might have been expected to have evolved higher affinity transport systems to acquire iron more effectively at low concentrations, as observed for other nutrients (for example Mn[11] and nitrate[13]). But Hudson and Morel[5] recently reported that Fe-uptake kinetics in two coastal phytoplankters approach the physical limits for diffusion of inorganic Fe species to the cell surface and for kinetics of Fe-ligand exchange at membrane transport sites. They predicted that oceanic algae could not have higher transport kinetics and that the only available means of adaptation to low-iron oceanic conditions would be a reduction in cell Fe requirement or size. A reduction in size does not apply in this case because clone 13-1 is larger than clone 3H; mean cell volumes were 139 ± 12 (±s.d.) and

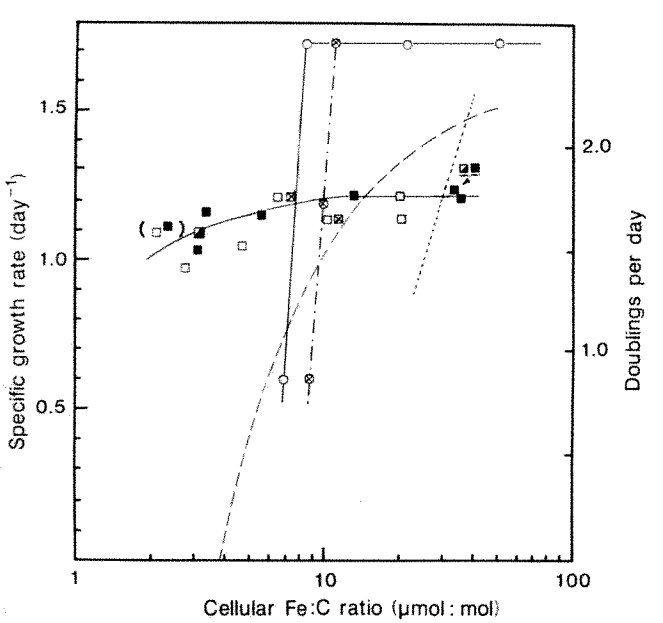

FIG. 2 Specific growth rate versus cell Fe:C ratio for a single 3H experiment based on intracellular (○) and total cell (⊗) Fe, for two 13-1 experiments based on intracellular Fe (◪, ■) and a third based on intra- (□) and total (⊠) cell Fe. Ratios of intra- to total cell Fe were 0.89 ± 0.005 and 0.77 ± 0.1 (±range, $n = 2$) for 13-1 and 3H at Fe concentrations ≤110 nM where $Fe(OH)_3$ does not precipitate. Data points in parentheses (■, □) are estimates for cultures without added Fe or ^{55}Fe, extrapolated from specific growth rate and relationships among cell Fe uptake rate, specific growth rate and Fe concentration (Figs 1 and 3; refs 11, 20). Cells were inoculated at 0.3 μmol cell C l^{-1} and grown for about 7 generations before measurement. Cell Fe levels were measured in narrow biomass range of 29–75 μmol cell C l^{-1} to have sufficient cell mass for accurate measurement without having enough to appreciably affect dissolved Fe chemistry or culture pH (ref. 5). Dashed line is data for *T. weissflogii* from Harrison and Morel[6] measured at 20 °C in continuous light normalized to cell C using our measured C:volume ratio of 5.6 mol l^{-1}. Dotted line is estimate of cell Fe:C growth requirement based on biochemical maximum use efficiency calculations of Raven[14] at 20 °C and saturating light. His specific growth rates computed for continuous light were multiplied by 0.58 to adjust to our 14:10 h light:dark cycle.

$32 \pm 4.7\ \mu m^3$, respectively. Our results confirm their predictions.

The amount of cellular Fe required for near maximum growth of clone 13-1 is much lower than minimum amounts thought necessary to meet the metabolic needs of plant cells[14]. It is ≤10% of calculated amounts needed for growth based on Fe enzymatic requirements in photosynthesis, respiration and NO_3 reduction (Fig. 2). It is also 10–100 times less than values previously used to estimate algal growth requirements for Fe in sea water [1,2]. These estimates were based on laboratory experiments with unacclimated cultures of the coastal species *T. weissflogii*[15] and on amounts of particulate Fe in near-surface sea water after subtracting amounts leachable by weak acid and estimated to occur in aluminosilicate minerals[2]. These latter values are uncertain as they do not correct for iron adsorbed to particles or present in iron oxides. On the basis of these values, it has been concluded that ratios of Fe:NO_3 in upwelling sea water were 10–100 times too low to meet the matabolic needs of phytoplankton and, therefore, that most of the Fe required for growth must be supplied from atmospheric deposition[1,2].

This conclusion needs to be reassessed in light of our findings. Dissolved Fe within the nutricline of the North Pacific is highly correlated with NO_3, PO_4 and SiO_3 concentrations[1,2], suggesting that it is regulated by biological uptake and regeneration processes as occurs for major nutrients. For this to be true, however, the relative changes in dissolved Fe and major nutrients with depth should reflect the concentrations of these elements in phytoplankton, the major biomass reservoir. Linear regressions between dissolved Fe and NO_3 in the nutriclines of four North Pacific stations[2] yield slopes of 13.5, 15.0, 11.3 and 13.8 μmol Fe:mol NO_3 (coefficient of variation, $r^2 = 0.98, 0.94, 0.96, 0.90$) which translate to a mean Fe:C ratio of 2.0 ± 0.2 μmol:mol, assuming a typical 6.6:1 C:N ratio in plankton[16]. This Fe:C ratio would support a specific growth rate of clone 13-1 of about 1.0 per day, suggesting that there could be enough dissolved Fe in deep water when it is advected to the surface to support low to moderate growth rates of oceanic species, assuming most of the Fe is biologically available.

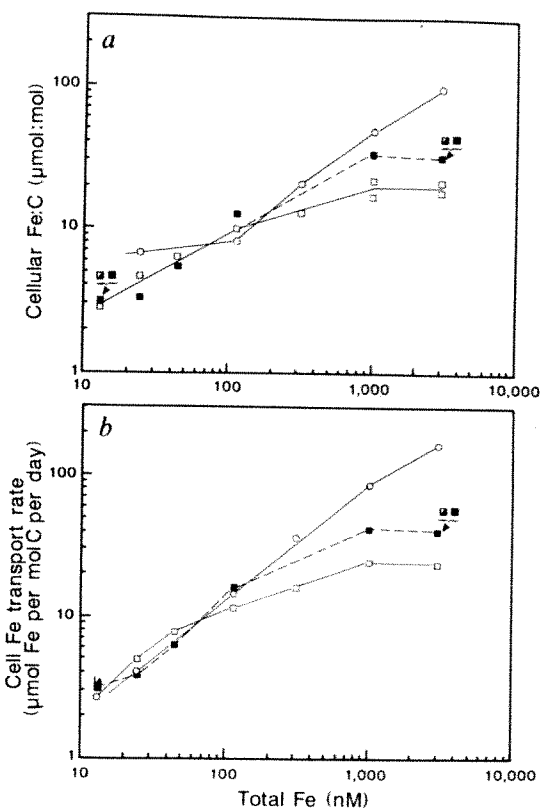

FIG. 3 *a*, Intracellular Fe:C ratios in clones 3H and 13-1 as functions of total Fe in medium. *b*, Specific Fe transport rates were computed by multiplying intracellular Fe:C ratios by specific growth rates. ○, 3H; ◪, ■ and □, three experiments with 13-1.

Although the Fe:C ratio derived from dissolved Fe to major nutrient correlations will support growth of the oceanic species, it is too low for growth of coastal diatoms *T. pseudonana* and *T. weissflogii* (Fig. 2). Thus, for a hypothetical algal community composed of these three species, the coastal diatoms would be selected against under oceanic low-Fe conditions and the community would be dominated by the slower growing oceanic diatom. Increasing the Fe concentration might have little immediate effect on community growth rate as the oceanic species might be growing near its maximum; but after a period the rate would increase as the community became dominated by the high-Fe species with faster maximum rates. This would explain observations from algal growth experiments in surface water from the subarctic Pacific[2,4] and Southern Ocean[3] in which Fe additions caused little or no effect for the first 2–4 days, but stimulated growth and caused associated shifts in species dominance after this period.

Data from the experiments in the subarctic Pacific[2] support our findings of the low cellular Fe:C growth requirements of oceanic algae. There was some growth in all of the controls (with no Fe added) in these experiments, despite low Fe concentrations in the water. At station T-6, for example, suspended particulate matter increased by 0.814 mg kg^{-1} (6.2-fold), which apparently was due to phytoplankton growth as it was accompanied by a 7.4-fold increase in chlorophyll. Dissolved Fe was 0.08 nM, and if all of it were taken up by the growing phytoplankton, we compute a maximum cellular Fe:C ratio of 2.6 μmol:mol (based on a 0.45 cellular C:dry weight ratio[14]). We compute a similar value (2.9 μmol:mol) if we base our growth estimates on the amount of NO_3 taken up by the cells (4.2 μM) and a C:N ratio for plankton of 6.6 (ref. 16). These estimates agree well with our growth requirements for clone 13-1 and with the Fe:C ratio (2.3 μmol:mol) derived from the relative increases in dissolved Fe and NO_3 within the nutriciline at this station as discussed above.

The results of our investigation, the covarying distributions of dissolved Fe and major nutrients, and the results of shipboard growth experiments all indicate that Fe is an important biologically controlling nutrient in the sea whose distribution is influenced by phytoplankton uptake and regeneration processes. Whether it is more important in controlling algal community growth rate and species composition than traditional major nutrients is unknown, and is currently being actively debated[17]. □

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Sexual selection and the potential reproductive rates of males and females

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PRONOUNCED sex differences in mating competition are a prominent feature of many animal breeding systems. These differences are widely attributed to sex differences in parental investment[1,2] which bias the ratio of sexually receptive females to males[3] (the operational sex ratio), generating more intense competition between members of one sex, usually males[3–5]. Unfortunately, relative parental investment[1] is usually impossible to measure in species where both sexes invest in their offspring[6,7] and there is currently no empirical basis for predicting the pattern of mating competition in these species. In contrast, the potential rate of reproduction by males and females (measured as the maximum number of independent offspring that parents can produce per unit time) is both more directly related to the operational sex ratio and more easily estimated in natural populations[7]. Here we show that among species where males care for the young, the sex with the higher potential reproductive rate competes more intensely for mates than the sex with the lower potential rate of reproduction.

In animals without parental care or where females are responsible for all care, the potential reproductive rate of males usually exceeds that of females. As a result, the operational sex ratio is biased towards males and males are the predominant competitors for mates[1,7,14], (except in a few cases where males contribute resources used in the production of zygotes[8–11]). In contrast, in species where males are responsible for all parental care while females pay the costs of egg production (which include some teleost fishes[14–16], anurans[17], urodeles[18], invertebrates[19,20] and a few birds[21,22]), the direction of mating competition differs between species. In some, females compete intensely for mates, males are choosey in selecting partners and females are brighter than males[23–26]. In others, males compete intensely for females, females are choosey in selecting partners, and males are brighter than females[27,28]. An explanation of these differences could be that only in some of these species does the involvement of males in parental care depress their potential reproductive rate below that of females[7].

To test whether differences in the direction of mating competition depend on which sex has the higher potential rate of reproduction, we extracted data on the maximal reproductive rates of males and females for 29 species where males were responsible for parental care, there was a clear sex difference in the intensity of mating competition, and data were available (Tables 1 and 2). With only two possible exceptions, males had potentially higher reproductive rates than females in all 'predominant male competitors' (Table 1). The most highly developed examples of predominant male competition combined with male parental care are found in fish and frogs where males can care for multiple clutches simultaneously or in quick succession[27–29]. For example, in the three-spined stickleback, *Gasterosteus aculeatus*, males can guard 10 or more clutches of eggs at a time and do so for about 2 weeks, whereas females can lay one clutch every 3–5 days[28]. Consequently, the potential reproductive rate of males is higher than that of females, the operational sex ratio is male-biased and males compete intensely for mates.

By contrast, in all species that we identified as 'predominant female competitors', females were able to achieve higher rates of reproduction than males. The clearest examples occur in small polyandrous shorebirds, where the potential reproductive rate of males is low because incubation is prolonged and brood size is small[21]. For example, in the polyandrous spotted sandpiper *Actitis macularia*, where females compete intensely for mating partners, males do not raise more than one clutch of four eggs during the breeding season, whereas females can produce an egg a day and lay clutches for up to four different males in the course of the season[23,30]. Predominant female competition also occurs in some fish where males carry eggs or young for lengthy periods and their reproductive rate is constrained by the number of eggs they can carry, including the pipefishes, *Nerophis ophidion* and *Syngnathus typhle*[25,26,31,32] and some cardinal fishes[33]. Further examples can be expected in other animals where males bear eggs or young.

TABLE 1 Relationship between reproductive rates and mating competition for species in which males are responsible for parental care

Female rate / Male rate	Competition for mates more intense in males	Competition for mates more intense in females
<1	Fish *Cottus* (2 spp) *Oxylebius pictus* *Chromis notata* *Chrysiptera cyanea* *Badis badis* *Pimephales promelas* *Etheostoma olmstedi* *Gasterosteus aculeatus* *Forsterygion varium* Frogs *Alytes obstetricans* *Hyla rosenbergii* *Eleutherodactylus coqui*	
>1	Fish *Hippocampus* spp Birds *? Rhea americana*	Fish *Apogon notatus* *Nerophis ophidion* *Syngnathus typhle* Birds *Actitis macularia* *Phalaropus* (2 spp) *Eudromias morinellus* *Jacana* (5 spp) *Rostrathula benghalensis* *Turnix sylvaticus*

Males compete more than females for access to mates in all but two of the species in which a male has a higher potential reproductive rate than a female (mainly ectotherms). Females are the more competitive sex (sex roles are 'reversed') in species where the potential reproductive rate of females exceeds that of males (primarily endotherms).

Both possible exceptions in Table 1 are instructive as they illustrate the need to calculate reproductive rates over different periods in different species. In the greater rhea, *Rhea americana*, males incubate broods of 20–30 eggs laid by several females and compete vigorously for mating access to female groups[36]. The potential reproductive rate of females calculated over the entire breeding season may be higher than that of males. During the period of mating and brood production however, males can fertilize and accept eggs faster than females can lay them and the operational sex ratio is probably male-biased. In seahorses (*Hippocampus* spp.), the operational sex ratio is biased towards males despite a prolonged male gestation period because the reproductive rate of females is constrained by monogamous pair bonds and by limited periods of receptivity[12].

The potential rates of reproduction by males and females thus provide a basis for predicting the direction of mating competition in the two sexes and thus the direction of sexual selection. Several other factors, however, can bias the operational sex ratio and influence the relative intensity of mating competition. These include behavioural adaptations to competition in the sex with the potentially higher reproductive rate, such as precopulatory guarding of multiple mates and earlier eclosion, emergence or arrival times[24,37–40]. Conversely, biases in the operational sex

TABLE 2 Maximum observed reproductive rates in species where males are responsible for parental care

Predominant male competitors

	Male care duration	Clutch (C) and brood (B) size	Interclutch interval	Max. F/max. M rate of reproduction	Competing sex	Ref.
Alytes obstetricans (Discoglossidae: Midwife toad)	2–3 weeks	M can carry > 1 clutch at a time	FF breed 2–4 times per summer at ~ monthly intervals	<1	M	45–47
Hyla rosenbergii (*Hylidae*)	4 days	B = 2,350	23 days	≪1 (0.25)	M	27
Eleutherodactylus coqui (Leptodactylidae)	17–26 days of care 8–9 month season	B = up to 5 × C at once C = 16–43	up to 6 clutches per season	<1	M	29
Cottus hangiongensis (Cottidae: river sculpin)		B = 3.4–5.3 × C up to 13 × C	2 per season	<1	M	49
Cottus gobio (Cottidae: river bullhead)	4 weeks	B = 2.15 × C C = 75–200	2 per season	<1	M	50
Oxylebius pictus (Hexagrammidae: greenling)	2.5–3.5 weeks 30 days between spawnings up to 7.5 × C per season	B = 0–10 × C per cycle B = 0–22 × C per season C = 1,500–5,000	3 per season	<1	M	51, 52
Chromis notata (Pomacentridae: damselfish)	4–12 days (depends on temperature) 8.2–17.8 days between spawnings	B = 1–4 × C 2.8–4.6 nests per season C = 10,000–27,500	7.2–18.4 days	<1	M*	15, 53
Chrysiptera cyanea (Pomacentridae: damselfish)	4 days MM spawn continuously	B = up to 12,255 eggs per cycle C = 900–2,500 eggs per cycle	4 days	<1	M	54
Badis badis (Nandidae)	2 days egg/3–4 days larvae 7–8 days between spawnings	B = 2–3 × C	4–7 days	<1	M	55, 56
Pimephales promelas (Cyprinidae)	4–8 days males spawn continuously for 3–5 weeks	B = max. 6,000 eggs per nest C = 200–700 eggs	3–4 days	<1	M	57, 58
Etheostoma olmstedi (Percidae: tessellated darter)	4 days	B = max. 2,000 eggs C = 19–324 (season $\bar{x}$ = 727)	5–16 days ($\bar{x}$ = 7.6)	≪1	M*	15, 59
Gasterosteus aculeatus (Gasterosteidae: stickleback)	2 weeks at 21 °C	B = up to 10 × C $B \gg C$ = 51–150	3–5 days	<1	M	28
Forsterygion varium (Tripterygidae)	7–10 days spawn up to 15 times per season	B = 20–7,080 ($\bar{x}$ = 2,245) eggs C = 20–1,680 ($\bar{x}$ = 796) eggs	100% spawn once 31% spawn > once never > 5 times per season	≪1 (0.3)	M	60
Hippocampus fuscus (Syngnathidae: Seahorse)	13–14 days	$C > B$	≈13–14 days	>1	M	12
Rhea americana (Greater Rhea)	incubation 36–37 days plus care one brood per season	B = 26.5 C = 2–3	up to 12 MM per year	eggs per season > 1 eggs per laying period < 1	M	34–36

Predominant female competitors

	Male care duration (including incubation)	Clutch (C) and brood (B) size	Interclutch interval	Max. F/max. M rate of reproduction	Competing sex	Ref.
Actitis macularia (Spotted sandpiper)	incubation 21 days max. 8.1 eggs per season usually one brood per season	$C = B$ = 4 1 egg per day	max. 11 eggs per season	>1	F	23, 30
Phalaropus lobatus (Red-necked phalarope)	incubation 17–21 days male care = 33 days usually one brood per season	$C = B$ = 4 1 egg per day	10 days up to 2 clutches per season	>1	F	24, 61
Phalaropus fulicarius (Grey phalarope)	incubation 18–20 days male care = 37 days usually one brood per season	$C = B$ = 4	up to 2 per season	>1	F	61, 62
Eudromias morinellus (Dotterel)	incubation 24–28 days male care = 61 days usually one brood per season	$C = B$ = 3	5–11 days up to 3 clutches per season	>1	F	61
Jacana spinosa (American jacana)	male care = 59 days total	$C = B$ = 4	up to 3 MM per year (≥4 clutches in few weeks')	>1	F	63
Jacana jacana (Wattled jacana)	about 60 days*	$C = B$ = 4 1 egg per day	minimum = 2–4 days up to 6 clutches per season (up to 2 MM)	>1	F	64, 65
Hydrophasianus chirurgus (Pheasant tailed jacana)	about 60 days*	$C = B$ = 4	several MM per F at same time	>1	F	66, 67
Metopidius indicus (Bronze winged jacana)	about 60 days*	$C = B$ = 4*	several MM per F at same time	>1	F	68
Actophilornis africana (African jacana)	incubation 24 days male care >60 days	$C = B$ = 4	up to 4 MM per F at same time	>1	F	69, 70
Rostrathula benghalensis (Painted snipe)	incubation 15–19 days 1–2 months care (62 days)	$C = B$ = 4	up to 4 MM per F at same time	>1	F	69
Turnix sylvaticus (Little button quail)	incubation 12–15 days/care 18–20 days male cycle = 53 days total	$C = B$ = 3.5	not known but estimated that F can breed with up to 5 MM per year	>1	F	69, 71
Apogon notatus (Apogonidae: cardinalfish)	8 days care plus 6–15 days = 14–23 days between spawning	$C = B$	10–19 days ($\bar{x}$ = 13.8)	>1	F	33
Nerophis ophidion (Syngnathidae: pipefish)	28–37 days	C = 1.8 × B M = 204 eggs per season F = 396 eggs per season		>1 (1.8)	F	25, 31
Syngnathus typhle (Syngnathidae: pipefish)	28–45 days	C = 1.9 × B M = 91 eggs per eason F = 219 eggs per season		>1 (1.9)	F	26, 31

Species shown are allocated to two categories on the basis of reports of mating competition: predominant male competitors (*a*) and predominant female competitors. (*b*) Although both sexes may compete for particular mating partners, most species could be allocated to the groups without difficulty. Records of reproductive rate ignore the possibility of 'stolen' copulations by males. Abbreviations: M, male; F, female; MM, males; FF, females. Columns show the best available estimates of: (1) the duration of male care of eggs (incubation) and/or young and of the time between successive broods where remating does not follow immediately on independence of the previous brood; (2) average clutch size laid by females (C) and the average brood size cared for by males (B). Where males are known to care for several clutches simultaneously, this is noted; (3) the approximate inter-clutch interval for females or the maximum number of successful breeding partners per season; (4) maximum recorded female reproductive rate (eggs per unit time) divided by the maximum recorded rate of reproduction by males (independent young reared per unit time). For most species, estimates of reproductive rate were only adequate to indicate whether the potential reproductive rate of males exceeded that of females or vice versa; (5) whether males or females are recorded as the primary competitors for mates or breeding territories. * In congenerics/other Jacana spp.

ratio may be reduced by sex differences in life expectancy[41], which can reflect the costs of increased competition in the potentially faster sex[7]. Variation in the time necessary to find mates may constrain mating competition in some species[42] (G. Parker, personal communication), while the form of competitive behaviour may be affected by variation in the costs and benefits of particular tactics to the two sexes. Finally, where the potential rate of reproduction is similar in the two sexes, the relative benefits of acquiring qualitatively superior mates[43,44], rather than the operational sex ratio, may determine the comparative intensity of mating competition in the two sexes. □

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Construction of a pattern-generating circuit with neurons of different networks

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RHYTHMIC motor behaviours are generated within the central nervous system by neuronal circuits called central pattern generators (CPG)[1]. Although a CPG can produce several forms of the same behaviour[2–5] and several circuits may interact to generate different behaviours[6], it is generally assumed that a given CPG consists of a predefined assemblage of neurons that is functionally distinguishable from other circuits. However, recent studies on the stomatogastric nervous system of crustacea have suggested that CPGs may not be immutable functional entities[7–10]. We now report that under an identified neuromodulatory stimulus, the CPG that produces swallowing-like behaviour of the foregut in lobsters is constructed *de novo* from neurons belonging to other CPGs. Consequently neurons operating independently as members of different circuits may be reconfigured into a new pattern-generating circuit that operates differently from the original circuits. This not only challenges the concept of the CPG being a discrete functional entity, but also demonstrates that a modulatory input can specify an appropriate CPG from a pool of individual neurons of diverse origins.

We performed our experiments on preparations *in vitro* of the stomatogastric nervous system (STNS) of the lobster *Homarus gammarus*. The STNS consists of four interconnected ganglia (Fig. 1*a*) that together generate well described motor rhythms of the four regions of the foregut[11]. These independent foregut rhythms control oesophageal ingestion of food, and its storage in the cardiac sac, trituration by the gastric mill system and filtration through the pylorus on the way to the midgut (Fig. 1*b*). We describe here a new distinct motor activity of the STNS which transfers food between these different foregut compartments. We show that this swallowing-like behaviour arises first from the rhythmic opening of a valve situated between the oesophagus and the cardiac sac (OCS valve) (Fig. 1*b*), and second from a massive reorganization of all other foregut rhythms (Figs 2 and 3).

In STNs preparations with the anterior part of the foregut left attached, the three dilator muscles (ocsv1–3; Fig. 1*b*) of the OCS valve are generally inactive and the latter remains closed. We have found that opening of the valve is driven from the commissural ganglion (Fig. 1*a*) by OCS dilator motoneurons, which in turn are controlled by two equivalent interneurons arising in the inferior ventricular nerve (Fig. 1*a*). These cells have been previously identified and named 'pyloric suppressors'[12] (PS). Intrasomatic depolarization of either PS neuron to evoke firing strongly activates ocsv dilator muscles (Fig. 1*c*). Although PS is generally silent *in vitro*, we believe the neuron has endogenous bursting properties that drive rhythmic dilation

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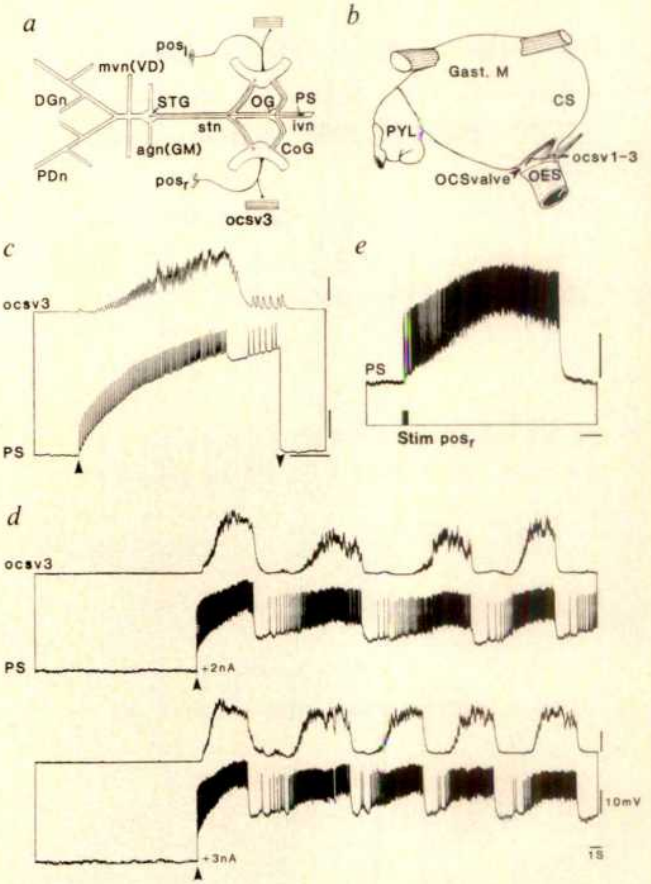

FIG. 1 Neuron pyloric suppressor (PS) drives rhythmic dilation of the oesophageal/cardiac sac valve (OCS) through its endogenous bursting properties. *a*, The isolated stomatogastric nervous system of *Homarus gammarus* showing the soma position and axonal projections of interneuron PS. *b*, Lateral view of the foregut showing the position of the OCS valve and the four functionally separate regions of the foregut. In *a* and *b*, anterior is to the right. *c*, Simultaneous intracellular recordings from the soma of PS and a fibre of oscv$_3$, a dilator muscle of the OCS valve. Discharge of neuron PS, here evoked by intrasomatic injection of depolarizing current (arrowheads), excites the dilator motoneuron to the OCS valve and causes synaptic depolarization of muscle oscv[3]. *d*, When continuously depolarized (current level 2 nA, upper panel; 3 nA, lower panel), an otherwise silent PS fires in repetitive spike bursts and rhythmically drives the dilator muscle of the OCS valve. The higher burst frequency in the lower panel indicates a voltage-dependent oscillatory mechanism intrinsic to PS itself. *e*, Sensory activation of neuron PS. A brief electrical stimulation (30 Hz for 250 ms) of the nerve branch to the right posterior oesophageal sensor (see *a*), a chemosensor in the internal wall of the OCS valve[14], triggers an active spike burst in PS that long outlasts the initial stimulus. Abbreviations: agn, anterior gastric nerve; CoG, commissural ganglion; CS, cardiac sac; DGn, nerve containing the axon of DG motoneuron; Gast. M, gastric mill; GM, gastric mill motoneuron; ivn, inferior ventricular nerve; mvn, median ventricular nerve; OCS valve, oesophageal-cardiac sac valve; ocsv1–3, dilator muscles 1–3 of the OCS valve; OG, oesophageal ganglion; OES, oesophagus; pos$_l$ and pos$_r$, left and right posterior oesophageal sensors; PDn, nerve containing the axon of PD motoneuron; PS, pyloric suppressor neuron; PYL, pyloric chamber; STG, stomatogastric ganglion; stn, stomatogastric nerve; VD, ventral dilator motoneuron. Dissections and electrophysiological recording and stimulation were made as previously described for this system[11].

of the OCS valve. First the cell always oscillates and generates repetitive bursts during occasional spontaneous bouts of activity, or in response to sustained depolarizing current (Fig. 1*d*). In either case, the ocsv dilator muscles are active in time with the bursts of PS. Second, bursting of PS is voltage-dependent, displaying a threshold for activation and an increase in frequency with increasing levels of tonic depolarization (Fig. 1*d*). Third, regenerative bursts in PS can be triggered synaptically by brief electrical stimulation of bilateral sensory nerves (Fig. 1*e*) from the posterior oesophageal sensors (Fig. 1*a*), also suggesting an input pathway appropriate for the induction of swallowing behaviour *in vivo*.

Neuron PS also makes synaptic connections with neurons of other STNS circuits; pyloric cells were previously shown to receive inputs from PS[13], and we have found similar direct connections onto gastric and oesophageal neurons (P.M., J.S. and M.M., manuscript in preparation). We therefore assessed the contribution of these individual circuits to swallowing behaviour. In contrast to PS neurons, all three circuits are continuously active *in vitro*. Figure 2*a* (left panel) shows spontaneous oesophageal (monitored by neuron OC) and pyloric (neuron VD) rhythms in the absence of PS activity. Figure 2*b* (left) shows a typical gastric rhythm (neurons GM, LG) recorded simultaneously with neurons VD and LP of the pyloric circuit.

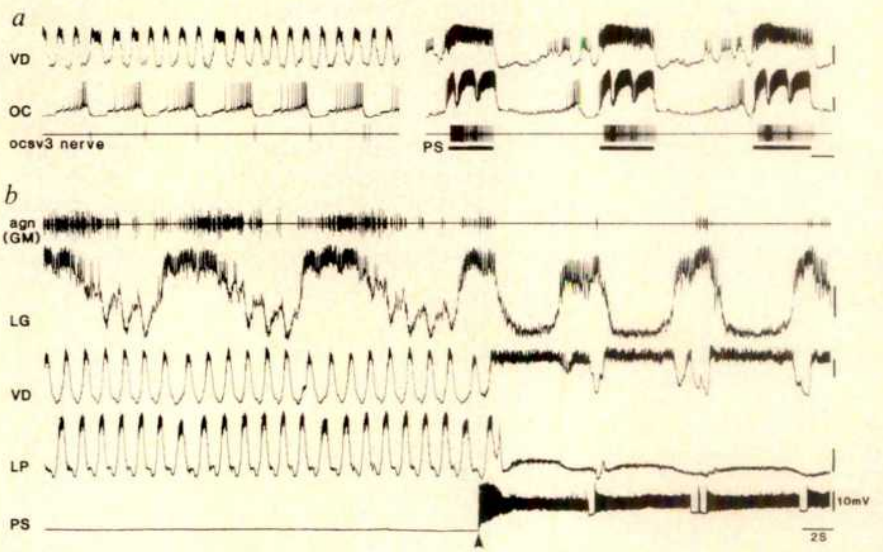

FIG. 2 Discharge of neuron PS restructures independent stomatogastric rhythms into a single novel motor pattern. *a*, left panel, Spontaneous pyloric and oesophageal circuit activity recorded simultaneously from neurons VD and OC, respectively. The oesophageal rhythm typically has a longer cycle period (4–6 s) than the pyloric rhythm (1–2 s). The extracellularly recorded dilator motoneuron to the OCS valve (oscv[3]) was weakly active in oesophageal time. *a*, right panel, Same preparation during spontaneous bursting in PS (cell not penetrated in this experiment; bars indicate timing of PS bursts). The OCS valve dilator motoneuron is now strongly activated by PS (as in Fig. 1*d*), and both VD and OC of the previously separate pyloric and oesophageal patterns become coordinated to this new rhythm. *b*, PS acts differently on individual stomatogastric neurons. Left, independent pyloric (VD, LP neurons) and gastric (LG, GM in agn) circuit activities in the absence of PS firing. The cycle period of the gastric rhythm is typically 10–15 s. When PS is strongly depolarized (arrowhead) and fires in long repetitive bursts, gastric LG and pyloric VD immediately combine to produce a new single rhythm in time with the interneuron, while activity in gastric GM and pyloric LP is immediately decreased or completely suppressed. Abbreviations: LG, lateral gastric motoneuron; LP, lateral pyloric motoneuron; OC, oesophageal constrictor motoneuron; other abbreviations as in legend to Fig. 1.

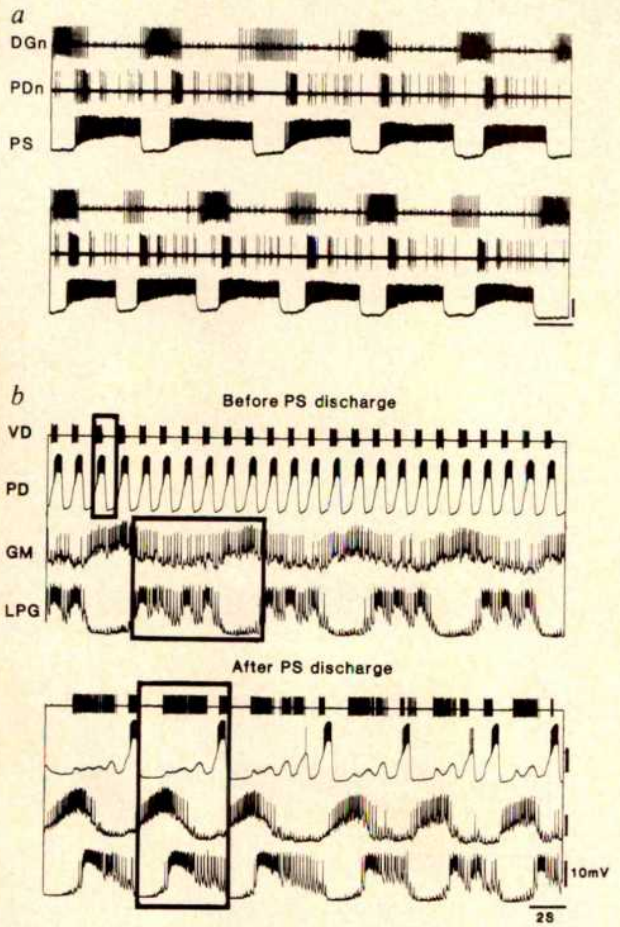

FIG. 3 Neuron PS imposes its own rhythm on the swallowing motor pattern and produces a long-lasting reconfiguration of stomatogastric circuits. *a*, PS-driven swallowing activity in the gastric and pyloric circuits monitored extracellularly from DG (DGn) and PD (PDn) motoneurons, respectively. The two panels show membrane potential oscillation and bursting in the same PS neuron in response to two different levels (3 nA, upper panel; 4 nA, lower panel) of tonic depolarizing current. The activity of both networks follow the voltage-dependent changes in rhythm frequency of PS. *b*, Simultaneous recordings from neurons of the pyloric (VD, PD) and gastric (GM, LPG) networks before and immediately after a 10 s burst (mean spike frequency 25 Hz) in PS. Upper panel, separate pyloric and gastric rhythms (rectangles) before PS firing. Lower panel, after PS discharge, the same neurons are coordinated in a single rhythm (rectangle) that is different from either of the two original patterns. Abbreviations: LPG, lateral posterior gastric motoneuron; PD, pyloric dilator motoneuron; other abbreviations as in legend to Fig. 1.

When the interneuron becomes active, however, dramatic alterations occur in these otherwise independent and very different motor patterns. First, the ongoing activity of all three circuits is reconfigured into a single novel pattern. The rhythms of neurons OC and VD become coordinated to spontaneous bursting in PS (here monitored by recording from the dilator nerve to the OCS valve; Fig. 2*a*, right panel); neuron VD now fires in single long bursts in time with PS while oesophageal constrictor neuron OC discharges rhythmically in high frequency bursts. Both cells remain inactive throughout most of the interneuron's interburst interval and the new rhythm bears no resemblance to either of the original oesophageal and pyloric rhythms. Similarly in Fig. 2*b* (right), current-induced bursting in PS produces a new combined pattern consisting of pyloric neuron VD firing in time with the interneuron, but alternately with the LG neuron, an integral member of the gastric circuit.

Second, neuron PS acts differently on individual elements of each circuit. Although certain neurons are strongly activated in phase (for example neurons VD and OC in Fig. 2*a*, right panel) or in antiphase (such as neuron LG and VD in Fig. 2*b*, right) with the interneuron, other cells are strongly inactivated (neurons GM and LP in Fig. 2*b*) and take no part in the swallowing motor pattern. Thus the interneuron can selectively discard integral members of different STNS circuits and assemble the remaining subpopulation of neurons into a new functional network.

Third, the PS neuron imposes its own rhythm on this swallowing network, thereby timing its activity to dilation of the OCS valve. This is evident in Fig. 3*a*, where PS is induced to burst at two different cycle frequencies by different levels of injected depolarizing current. In each case (Fig. 3*a*, upper and lower panels) activity in gastric neuron DG and pyloric neuron PD, like the dilator motoneuron of the OCS valve (Fig. 1*d*), follow closely the inherent rhythm of PS.

Fourth, neuron PS exerts long-lasting influences on STNS activity (Fig. 3*b*). Although original oesophageal rhythmicity returns soon after PS discharge (not shown), neurons ordinarily belonging to the gastric (GM, LPG) and pyloric (VD, PD) networks (Fig. 3*b*, upper panel) remain coordinated in a single pattern (Fig. 3*b* lower panel) for further tens of seconds before they again recover their separate gastric and pyloric network identities.

This reconfiguration of entire foregut motor activity seems to be behaviourally relevant. Our data imply that when the OCS valve dilates cyclically *in vivo* (during an episode of PS bursting; Fig. 1*d*): (1) a strong increase in oesophageal rhythmicity during each opening of the valve (Fig. 2*a*, right panel) serves to push food into the stomach; and (2) a unified cycling pattern, also synchronized to valve openings, occurring in the more posterior regions of the foregut (Fig. 2*b* and 3*a*), facilitates the rearward transit of the food. This cooperative swallowing pattern persists for some time after closure of the OCS valve (PS neuron again silent; Fig. 3*b* lower panel), before the flow of ingested food terminates and each foregut region resumes its separate motor function.

The effects of PS on individual pyloric, oesophageal and gastric neurons suggest several mechanisms by which the cell reconfigures the various STNS networks. First, PS modulates intrinsic membrane characteristics in several target neurons (such as causing the long-term suppression of oscillatory properties in pyloric cell LP (ref. 13; Fig. 2*b*) and so considerably

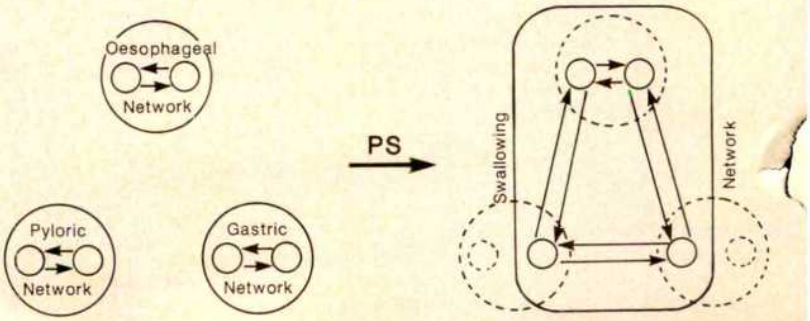

FIG. 4 Diagrammatic representation of PS-induced construction of the swallowing neural network from individual circuits of the stomatogastric nervous system. Left; with no PS activity, the oesophageal, pyloric and gastric pattern generators operate independently at significantly different cycle periods and control their respective regions of the foregut. Right; rhythmic discharge of PS drives opening of the OCS valve and, by breaking down the pyloric and gastric circuits and superimposing its own rhythm, the interneuron builds a single new network appropriate for swallowing behaviour.

disrupts the ability of the parent network to produce its inherent rhythm. Second, PS also acts directly on many oesophageal, gastric and pyloric neurons by way of conventional excitatory and inhibitory synapses[13], serving to override remaining intranetwork interactions and couple these elements to the interneuron's own rhythm (Figs 2 and 3*a*). It is also probable that the neuron PS modulates and potentiates normally weak synaptic interactions[9], such as those between pyloric and gastric neurons[11], thereby explaining the coordination of these networks that persists after the interneuron falls silent (Fig. 3*b*).

Figure 4 summarizes these results which show that expression of a behaviour can be obtained by a complete restructuring of several networks that otherwise produce different behaviours. Although different patterns of motor output may arise from changes within individual neural networks[3,4], only recently has such functional reconfiguration been found to extend to apparently separate networks composed of different neurons. In lobster and crab STNS, individual neurons can switch between different rhythm-generating networks[7,8,10] and two independent networks can merge into a single functional unit[9]. In our study, rather than just a switch in neuronal 'allegiance' to two ongoing rhythms, or 'fusion' of whole active networks to produce a combined rhythm, we find the selective dismantling of preexisting networks and the construction of a completely new motor circuit. This indicates that a pattern-generating network may exist only in a particular behavioural situation dictated by neuromodulatory influences. □

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Mutant α subunits of G_{i2} inhibit cyclic AMP accumulation

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ONE or more of three G_i proteins, G_{i1-3}, mediates hormonal inhibition of adenylyl cyclase[1–3]. Whether this inhibition is mediated by the α or by the $\beta\gamma$ subunits of G_i proteins is unclear[1,2]. Mutations inhibiting the intrinsic GTPase activity of another G protein, the stimulatory regulator of adenylyl cyclase (G_s), constitutively activate it by replacing either of two conserved amino acids in its α subunit (α_s)[4–7]. These mutations create the *gsp* oncogene which is found in human pituitary and thyroid tumours[5,8]. In a second group of human endocrine tumours, somatic mutations in the α subunit of G_{i2} replace a residue cognate to one of those affected by *gsp* mutations[8]. This implies that the mutations convert the α_{i2} gene into a dominantly acting oncogene, called *gip*2 (ref. 8), and that the mutant α_{i2} subunits are constitutively active. We have therefore assessed cyclic AMP accumulation in cultured cells which stably or transiently express exogenous wild-type α_{i2} complementary DNA or either of two mutant α_{i2} cDNAs. The results show that putatively oncogenic mutations in α_{i2} constitutively activate the protein's ability to inhibit cAMP accumulation.

We mutated mouse α_{i2} at codons cognate to either of the two α_s codons affected by *gsp* mutations[5,8], producing cDNAs that encode leucine instead of glutamine at position 205 (α_{i2}-Q205L) or cysteine instead of arginine at position 179 (α_{i2}-R179C). Wild-type (WT) and mutant G protein α chains were stably expressed in NIH3T3 cells using retroviral infection with a vector containing a dominant selectable marker, *neo*, which confers resistance to G418. Infected G418-resistant pools were tested for cAMP accumulation in response to prostaglandin E_1 (PGE_1), which stimulates adenylyl cyclase by way of receptors coupled to G_s, or to forskolin, which is thought to stimulate adenylyl cyclase directly[9]. In control cells (infected with vector alone or with vectors expressing α_s-WT or α_{i2}-WT), PGE_1 and forskolin elevated cellular cAMP by about 4- and 20-fold, respectively (Table 1). As in other cells[10,11], lysophosphatidic acid (LPA) inhibited PGE_1- and forskolin-stimulated cAMP accumulation by 30–50% in all the control populations of NIH-3T3 cells (Table 1). Treatment with pertussis toxin (PTX; 100 ng ml^{-1} for 20 h) abolished the inhibitory effect of LPA (result not shown), suggesting that it is mediated by a G_i-like protein.

Mutationally activated α_s (α_s-Q227L) constitutively increased basal and stimulated cAMP accumulation (Table 1; refs 5 and 12). Expression of the two mutant α_{i2} subunits also produced a dominant, constitutive effect on cAMP accumulation, albeit in the opposite direction. Expression of either α_{i2}-R179C or

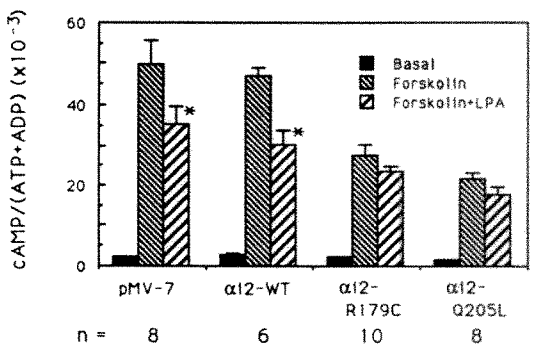

FIG. 1 cAMP accumulation in multiple individual clones derived from G418-selected pools of NIH3T3 cells infected with vector (pMV-7), or vector containing α_{i2}-WT, α_{i2}-Q205L, or α_{i2}-R179C. Clonal lines were generated by limiting dilution and cAMP accumulation was measured as described in the legend to Table 1. Forskolin was used at 50 μM, LPA at 100 μM. The results represent the mean ±s.e.m. of the responses obtained from the number of clones indicated in parentheses. *LPA significantly reduced the forskolin-stimulated activity; paired *t*-test, $P < 0.05$.

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TABLE 1 cAMP accumulation in pools of infected NIH-3T3 cells

	cAMP/(ATP + ADP)($\times 10^{-3}$)				
	Basal	PGE_1*	PGE_1 + LPA†	Forskolin*	Forskolin + LPA†
NIH-3T3	1.8 ± 0.1	6.6 ± 0.2	4.4 ± 0.35	47.6 ± 2.0	30.8 ± 1.3
pMV-7	2.0 ± 0.2	7.9 ± 0.4	4.5 ± 0.4	47.4 ± 5.5	31.0 ± 2.2
α_s-WT	2.1 ± 0.1	7.2 ± 0.4	4.1 ± 0.3	45.5 ± 2.7	29.4 ± 3.9
α_s-Q227L	4.2 ± 0.7‡	10.0 ± 1.4	8.1 ± 0.9§	135 ± 15.3‡	91.1 ± 12.1
α_{i2}-WT	1.7 ± 0.1	6.3 ± 0.1	4.5 ± 0.3	46.0 ± 0.4	32.1 ± 2.0
α_{i2}-Q205L	1.5 ± 0.1	3.1 ± 0.4‡	2.6 ± 0.2§	21.3 ± 1.8‡	17.6 ± 1.4§
α_{i2}-R179C	1.7 ± 0.1	4.8 ± 0.2‡	3.2 ± 0.1§	31.7 ± 3.1‡	24.4 ± 4.6§

[^{3}H]cAMP in [^{3}H]adenine-labelled NIH3T3 fibroblasts infected with either vector alone or vectors containing the indicated cDNA was estimated by determining the ratios of cAMP to total ATP and ADP pools. PGE_1 and forskolin were present at 50 μM, LPA at 100 μM. Values represent the mean ±s.e.m. of 3–5 independent experiments in triplicate determinations. Mouse α_{i2} cDNA[26] was subjected to site-directed mutagenesis, using the Bio-Rad Muta-Gene kit. Wild-type and mutant cDNAs were subcloned into the retroviral vector pMV-7, processed through packaging cell lines and used to infect NIH-3T3 cells, as described[27]. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum and infectants were selected in the same medium supplemented with 500 μg per ml G418. For measuring intracellular cAMP, confluent cells in 24-well plates prelabelled with [^{3}H]adenine (2 μCi ml^{-1} for 20–24 h) were washed once with HEPES-buffered DMEM and incubated (37 °C for 30 min) in the same medium containing 1 mM 1-methyl-3-isobutylxanthine with or without the indicated drugs. Reactions were terminated by aspiration and the immediate addition of 5% trichloroacetic acid (1 ml per well). Acid-soluble nucleotides were separated on ion-exchange columns as described[28].

* Stimulatory responses to PGE_1 or forskolin were significantly higher than the corresponding basal values; paired *t*-test, $P < 0.05$.

† Unless otherwise stated, responses to PGE_1 or forskolin were significantly inhibited in the presence of LPA; paired *t*-test, $P < 0.05$.

‡ Significantly different from uninfected or vector infected cells; *t*-test, $P < 0.05$.

§ Inhibitory responses produced by LPA were statistically insignificant; *t*-test.

α_{i2}-Q205L decreased both PGE_1- and forskolin-stimulated cAMP accumulation in pools of G418-resistant cells (Table 1) and in multiple individual clones generated from pools of infected G418 resistant cells (Fig. 1); α_{i2}-WT did not affect cAMP accumulation.

The inhibitory effects of α_{i2}-R179C and α_{i2}-Q205L lowered agonist-stimulated cAMP accumulation to levels at or below those produced by the combination of stimulatory agonist plus LPA in control cells, suggesting that both mutations constitutively activate α_{i2}. Inhibition by mutationally activated α_{i2} seems to mimic G_i-mediated inhibition of cAMP accumulation by LPA; the two inhibitory effects are not additive (Table 1; Fig. 1).

To extend this investigation, we examined hormonal regulation of cAMP accumulation in a human embryo kidney cell line, 293, cotransfected with the cDNA to be tested and with a vector expressing the cDNA for the rat luteinizing hormone receptor (LHR)[13], which stimulates adenylyl cyclase through G_s. Cotransfection with cDNA encoding the porcine α_2 adrenoceptor[14] allowed an α_2 adrenoceptor agonist, UK-14304, to reduce the cAMP accumulation stimulated by an LHR agonist, human chorionic gonadotropin (hCG), by 60% (Fig. 2*a*). The effect of UK-14304, like that of hCG, required expression of the appropriate receptor. Therefore, the stimulatory and inhibitory receptors are expressed in the same subpopulation of 293 cells.

In this model system, transient expression of either α_{i2}-R179C or α_{i2}-Q205L inhibited hCG-stimulated cAMP accumulation. These inhibitory effects increased with increasing mutant α_{i2} DNA (Fig. 2*b*) and were similar to those mediated by the α_2 adrenoceptor. Inhibition by mutant α_{i2} was not additive to that mediated by the α_2 adrenoceptor (result not shown). Although PTX prevented inhibition of cAMP accumulation by UK-14304 and LPA in 293 and NIH3T3 cells, respectively, the toxin did not affect inhibition of cAMP accumulation by mutant α_{i2} in either 293 (Fig. 2*b*) or NIH3T3 cells (results not shown). The mutant α_{i2} chains were nonetheless good substrates for PTX-catalysed ADP-ribosylation (Fig. 3), consistent with observations[2,15,16] that covalent modification by PTX preferentially impairs the interactions of α subunits with receptors, rather than effectors.

Effects of two control DNAs, encoding bovine prolactin or α_{i2}-WT, indicated that the inhibitory effects of mutant α_{i2} were specific in 293 cells (Fig. 2*b*). α_{i2}-WT inhibited cAMP accumulation only at the highest DNA concentration tested.

Our observations in NIH3T3 and 293 cells indicate that α_{i2} mutations designed to mimic GTPase-inhibiting mutations of α_s constitutively activate the protein, so that cAMP accumulation is inhibited. These results reinforce the view that G protein α chains use similar three-dimensional structures and the same molecular mechanism to hydrolyse GTP. Specifically, this probably involves the side chains of two conserved amino acids in all α chains: A glutamine equivalent to positions 205 of α_{i2} or 227 of α_s and an arginine equivalent to positions 179 and 201 of the same proteins.

Others[1,17] have proposed that G_i mediates hormonal inhibi-

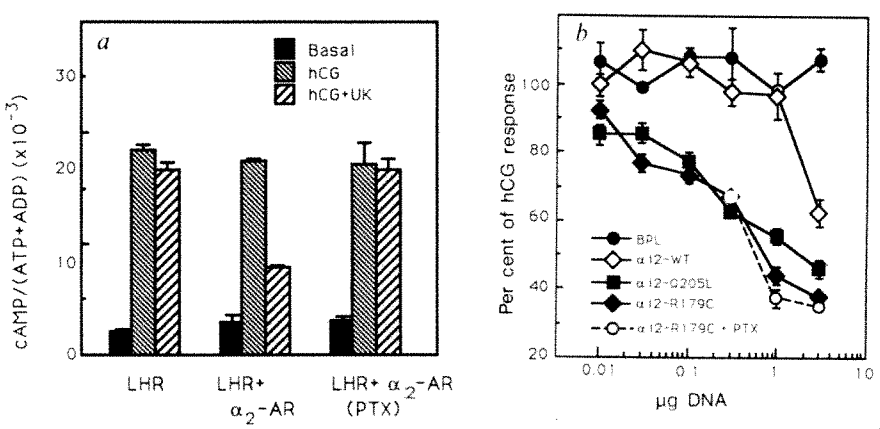

FIG. 2 cAMP accumulation in 293 cells. *a*, Inhibition of the stimulatory effect of hCG (5 ng ml^{-1}) by an α_2 adrenoceptor agonist, UK-14304 (10 nM), in cells transfected with DNA encoding LHR (0.5 μg DNA per 10^6 cells), or LHR plus α_2 adrenoceptor (α_2-AR; 0.05 μg DNA per 10^6 cells). *b*, Inhibition of hCG-stimulated cAMP accumulation by wild-type and mutant α_{i2} in cells cotransfected with DNA containing LHR (0.5 μg DNA per 10^6 cells) and the indicated amounts of DNA containing α_{i2}-WT, α_{i2}-Q205L, α_{i2}-R179C, or bovine prolactin (BPL). Results are expressed as a per cent of the hCG-stimulated (5 ng ml^{-1}) activity as compared with that measured in cells transfected with LHR alone. The data represent triplicate determinations in one experiment; two additional experiments gave similar results.

METHODS. Cells were maintained in DMEM containing 10% fetal calf serum. Transient expression of LHR and α_2 adrenoceptor cDNAs in the vectors pCIS and pCMV4, respectively, has been described[13,14]. Wild-type and mutant α_{i2} cDNAs were subcloned into the eukaryotic expression vector pcDNA1 (Invitrogen) and transfected into 293 cells (10^6 cells on 60-mm culture dish) by calcium phosphate precipitation. Sixteen hours after transfection, cells were rinsed once with Waymouth's medium containing 1 mg per ml BSA and re-seeded in 12-well plates. Cells were then labelled with [^{3}H]adenine for 24 h, in the absence or presence of PTX (100 ng ml^{-1}), and cAMP accumulation was assayed (48 h after transfection) in the presence of 1 mM 1-methyl-3-isobutylxanthine and the indicated drugs as described in the legend to Table 1.

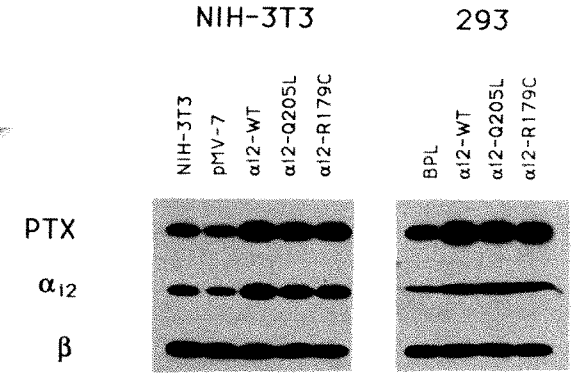

FIG. 3 Detection of α_{i2} and $\beta\gamma$ subunits in cells expressing wild-type and mutant α_{i2}. Membrane preparations were subjected either to immunoblot analysis using antisera specific to α_{i2} or $\beta\gamma$ subunits, or to [^{32}P]ADP-ribosylation catalysed by PTX. Membranes were prepared from representative clones of NIH3T3 cells isolated from pooled cell populations infected with the indicated DNA (left) or from transfected 293 cells (right). The 293 cells were transfected with 0.5 μg LHR DNA and 0.3 μg α_{i2} or control DNA (encoding bovine prolactin) per 10^6cells.

METHODS. Cells were frozen and thawed once and then homogenized by 10 passages through a 27-gauge needle. Nuclei were removed by low-speed centrifugation and a membrane fraction isolated by centrifugation for 30 min in a microfuge. Membrane proteins (100 μg) were resolved on 12.5% SDS-polyacrylamide gels and transferred to Immobilon-P membranes (Millipore). Detection of the antibody–antigen complex was by ^{125}I-labelled protein A. Rabbit polyclonal antibodies were raised against the α_{i2} peptide sequence CAAEEQGMLPEDLSG (residues 112–126) and the specificity confirmed by immunoblotting of purified α_i subunits isolated from bovine brain or human platelets (data not shown). The antisera were affinity-purified before use. The specificity of antiserum Beta-8 for the detection of $\beta\gamma$ subunits was as previously described[29]. PTX-catalysed [^{32}P]ADP-ribosylation reactions using 50 μg membrane protein were performed exactly as described[30]. Films were exposed for 2–3 days for immunoblots, and 2–4 h for ADP-ribosylation.

tion of adenylyl cyclase by a mechanism in which $\beta\gamma$ first dissociates from α_i and then binds to and inactivates α_s. This is consistent with the ability of $\beta\gamma$ to inhibit α_s (ref. 18) and adenylyl cyclase activity *in vitro*. But other evidence[16,19,20] suggests that α_i can directly inhibit adenylyl cyclase, without help from $\beta\gamma$. Moreover, titration of α_s by $\beta\gamma$ cannot be the sole mechanism by which G_i mediates hormonal inhibition of adenylyl cyclase, because GTP analogues and somatostatin inhibit adenylyl cyclase in mutant S49 lymphoma cells totally lacking α_s (refs 21–25).

Although the $\beta\gamma$ subunit of G_i may mediate hormonal inhibition of adenylyl cyclase in certain circumstances, inhibition of cAMP accumulation in our experiments is difficult to explain as an effect of $\beta\gamma$ and is much more likely to be mediated by α_{i2}. The amount of immunoreactive β does not detectably increase in either NIH3T3 or 293 cells expressing exogenous α_{i2}, whereas the 'extra' mutant (or wild-type) α_{i2} can easily be detected by immunoblots and PTX-catalysed ADP-ribosylation (Fig. 3).

Our experiments show that mutations in the glutamine-205 and arginine-179 codons of α_{i2} produce a protein that constitutively inhibits cAMP accumulation in cultured cells. Although the mechanism of this inhibition has not been determined, it probably involves constitutive activation of the α_{i2} chain. These conclusions support the proposal[8] that α_{i2} mutations create a dominantly acting *gip*2 oncogene, although they do not necessarily imply that decreased cAMP accumulation mediates the oncogenic effect of *gip*2. □

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Early aspects of *Caenorhabditis elegans* sex determination and dosage compensation are regulated by a zinc-finger protein

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THE *sdc*-1 gene acts at an early step in the regulatory hierarchy that controls the choice of sexual fate in *Caenorhabditis elegans*. It functions at a point before the control of sex determination and X-chromosome dosage compensation diverge. Here we report that *sdc-1* encodes a protein of 1,203 amino acids containing seven zinc fingers. This protein motif in combination with other genetic and molecular information suggests that *sdc-1* is likely to function as an embryonic transcription factor regulating downstream genes involved specifically in the sex determination and dosage compensation pathways, or regulating other genes involved in the coordinate control of both processes. These results enhance our general understanding of sex determination strategies, which are already known to involve transcriptional regulation[1] and alternative RNA splicing[2,3] in *Drosophila melanogaster*, DNA rearrangements in *Saccharomyces cerevisiae*[4], and transcriptional regulation in mammals[5,6].

In *C. elegans*, the primary sex-determining signal, the X/A ratio (ratio of X chromosomes to autosomal sets), controls sex determination and dosage compensation through its effects on genes that coordinately control both processes[7–12] (Fig. 1). Until now nothing was known regarding the molecular nature of the genes that respond most directly to the X/A ratio and act early in the regulatory hierarchy to control both sex determination and dosage compensation. The *sdc-1* gene (sex determination and dosage compensation) is among the genes required in hermaphrodites (XX) to permit the correct choice of sexual fate and level of X chromosome expression. Mutations in *sdc-1* have no effect in males (XO), but in XX animals result in masculinization (Tra phenotype), reflecting a defect in sex determination, and dumpiness (Dpy), reflecting the elevated X-linked

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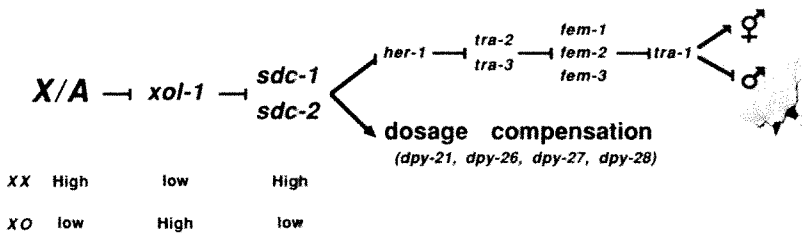

FIG. 1 Regulatory network controlling *C. elegans* somatic sex determination and dosage compensation[7]. The genes *xol-1*, *sdc-1* and *sdc-2* are involved in the coordinate control of sex determination and dosage compensation. The branch of the pathway involved only in sex determination is composed of the *her-1* and *fem* genes, which are required for male sexual development[8], and the *tra* genes, which are required for hermaphrodite development[8]. The genes that act downstream to implement dosage compensation include *dpy-21*, *dpy-26*, *dpy-27* and *dpy-28*, which are thought to reduce expression of each X chromosome in XX animals[33,36]. Mutations in these genes result in overexpression of X-linked genes and a Dpy phenotype in XX animals; mutations in all but *dpy-21* result in a maternal-effect XX-specific lethality[33]. The *sdc* genes control the hermaphrodite modes of sex determination and dosage compensation and act as negative regulators of the *her-1* sex determination gene and positive regulators of the XX-specific dosage compensation genes[9-11]. Mutations in either *sdc-1* or *sdc-2* cause XX animals to adopt the male mode of both processes, resulting in masculinization and overexpression of X-linked genes. Unlike *sdc-2* mutations, *sdc-1* mutations exhibit strong maternal rescue but do not result in significant XX-specific lethality. The gene *xol-1* (for 'XO-lethal') is required in XO animals to control the male modes of sex determination and dosage compensation. Mutations in *xol-1* cause XO animals to adopt the hermaphrodite modes of both processes, resulting in feminization, reduced X-linked transcript levels and XO-specific lethality. Mutations in *sdc-1* or *sdc-2* suppress the phenotypes caused by *xol-1* mutations, indicating that in XO animals the wild-type role of *xol-1* is to act as a negative regulator of the *sdc* genes[12]. Arrows represent positive regulatory interactions; bars represent negative interactions. 'High' and 'low' represent the activity states of the various genes in hermaphrodites (XX) and males (XO).

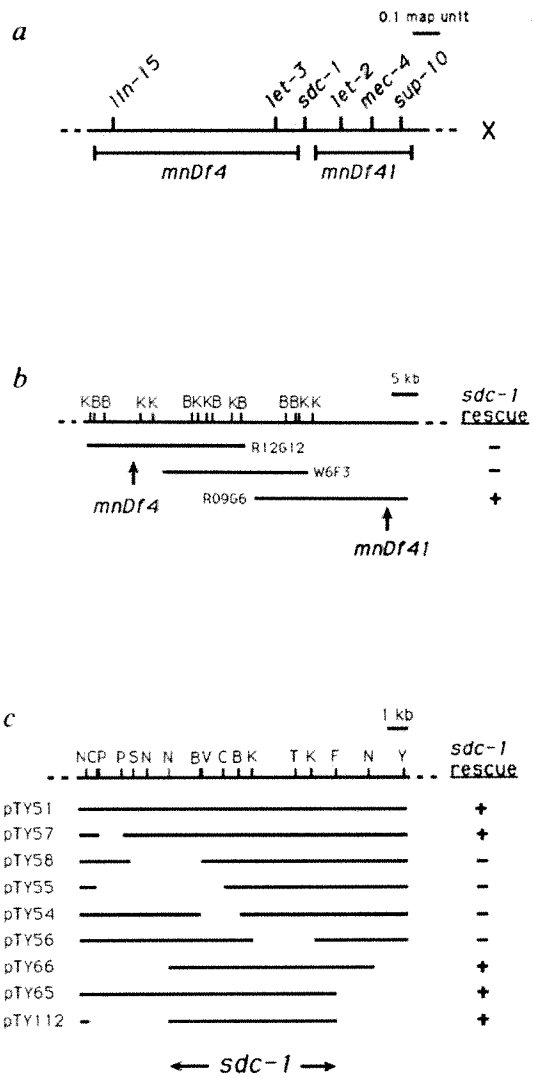

FIG. 2 Genetic and physical maps of the *sdc-1* region on the X chromosome. *a*, A genetic map of the right arm of the *C. elegans* X chromosome[10]. The genetically determined extent of chromosomal deficiencies *mnDf4 and mnDf41*, which flank *sdc-1*, are drawn below the map. The right breakpoint of *mnDf4* was previously positioned left of *let-3* (ref. 34), but an X chromosome carrying this deficiency does not complement *let-3* in our experiments. *b*, Localization of the *sdc-1* rescuing activity within an interval defined by deficiency breakpoints. A restriction map of a 65-kb genomic interval derived from restriction analysis of three overlapping cosmid clones is shown. The approximate positions of the right breakpoint of *mnDf4* and left breakpoint of *mnDf41* are indicated by arrows. Each cosmid in the interval was assayed by germ-line transformation for its ability to rescue the dumpy (Dpy), egg-laying (Egl) and sexually transformed (Tra) phenotypes of *sdc-1* mutants when incorporated into a stably transmitted extrachromosomal array. *c*, An expanded restriction map of the left half of the rescuing cosmid R09G6 and the structure of subclones of this region. Each subclone was tested for its ability to rescue an *sdc-1* mutant. The *sdc-1* rescuing activity resides in a 9-kb *Nhe*I–*Sfi*I interval. Plasmid pTY51 is a R09G6 derivative that carries a 14-kb *Bst*EII fragment deletion. The remainder of the subclones are pTY51 derivatives that contain deletions of the restriction fragments indicated by the open space. Plasmid pTY112 removes 4.1 kb of *Nhe*I genomic fragments from pTY65. Restriction site abbreviations are *Bam*HI (B), *Cla*I (C), *Kpn*I (K), *Nhe*I (N), *Not*I (T), *Pst*I (P), *Pvu*II (V), *Sfi*I (F), *Stu*I (S), *Bst*EII (Y). Southern hybridization experiments using probes that span the entire gene indicated that the 14 *sdc-1* alleles cause no obvious allele-specific polymorphisms. METHODS. To localize the deficiency breakpoints, cosmids at various distances from *sup-10* (positioned on the map by C. Cummins, J. Levin, P. Anderson & R. Horvitz, personal communication) were used to probe Southern blots of DNA derived from a wild-type N2 strain and strains homozygous for the *mnDf4 or mnDf41* deficiency and heterozygous for duplication (*mnDp1/+; mnDf4 and mnDp1/+; mnDf*41). Genomic fragments located outside the deficiency should be present in three copies, and those under the deficiency should be present in one copy. We localized the *mnDf4* right breakpoint to a 4-kb *Pst*I–*Stu*I fragment within cosmid R12G12 and the left breakpoint of *mnDf41* to a 3.5-kb *Hin*dIII fragment in R09G6. The position of the *sdc-1* gene was further localized by germ-line transformation rescue assays[35]. Test DNA (20 $\mu g\ ml^{-1}$) and the dominant *rol-6(su1006)* roller marker (pRF4 plasmid at 100 $\mu g\ ml^{-1}$) were co-injected into the syncytial gonad of young adult *sdc-1 (y67ts)* (ref. 10) animals grown at 15 °C. Injected animals were self-fertilized at 25 °C. F_1-transformed roller progeny carrying extrachromosomal arrays were scored for dumpy (Dpy), egg-laying defective (Egl) and sexually transformed (Tra) phenotypes, picked and self-fertilized. The F_2 broods were screened for the presence of roller animals. Most F_1 transformants are unstable and do not transmit the extrachromosomal arrays to their progeny. 'Stably' transformed lines were established from animals that transmitted the roller marked array to their progeny (5–80% transmission). A line was considered (+) in the rescue assay if both transient F_1 roller animals and stably transformed roller animals were rescued for Dpy, Egl and Tra phenotypes. A few clones (pTY54, pTY55, and pTY58) were capable of partially rescuing the *sdc-1* phenotype in the F_1 generation, but stable transformants carrying these plasmids were never rescued. Arrays of both pTY66 and pTY65 were shown to rescue *sdc-1(y36am)* (ref. 10). Plasmid pJS328 (gift of J. Shaw) contains a 2.2-kb *Bgl*II–*Hin*dIII fragment of the *act-4* promoter and first intron inserted in pBluescript KS(+) and pTY59 contains a 330-bp *Bgl*II–*Eco*RI fragment of the *act-4* first intron inserted into pBluescript KS(+). The *act-4* first intron is the location of one of the X chromosome elements that ferminizes animals with intermediate X/A ratios[15]. None of 50 *sdc-1* mutant animals transformed with pJS328 were suppressed; none of 240 *sdc-1* mutant animals transformed with pTY59 were suppressed.

transcript levels caused by a disruption of dosage compensation. Genetic experiments indicate that *sdc-1* functions directly or indirectly to negatively regulate the *her-1* sex determination gene, which is required for male development, and to activate the downstream XX-specific genes required for proper dosage compensation[9,10] (Fig. 1).

We have cloned *sdc-1* using a physical map of the *C. elegans* genome consisting of groups of overlapping cosmid and yeast artificial chromosome clones (refs 13 and 14; A. Coulson and J. Sulston, personal communication). The *sdc-1* gene is located on the right side of the X chromosome (Fig. 2*a*) in a region represented on the physical map by a series of overlapping cosmids spanning about 3 megabases, from the *sup-10* gene towards *sdc-1*. Cosmid probes positioned on this physical map were used to search for the breakpoints of two deficiencies that flank *sdc-1* (Fig. 2*a* and *b*). The deficiency breakpoints localized the *sdc-1* gene to a 65-kilobase (kb) interval (Fig. 2*b*).

To confirm the position of the *sdc-1* gene in this region, we used germ-line transformation to rescue an *sdc-1* temperature-sensitive mutant. Of the three cosmids that span the *sdc-1* region, only a single cosmid, R09G6, was capable of rescuing the *sdc-1* mutant phenotypes (Fig. 2*b*). A series of R09G6 subclones was constructed and tested for the ability to rescue the *sdc-1* mutant phenotypes (Fig. 2*c*). These experiments localized the *sdc-1* rescuing activity to a 9-kb *Nhe*I–*Sfi*I genomic DNA fragment. This fragment also rescues the mutant phenotype of animals carrying a putative null amber allele of *sdc-1*.

Genetic experiments indicate that large X chromosome duplications that do not cover *sdc-1* can feminize *sdc-1* mutant animals and thus suppress the *sdc-1* phenotypes[10]. We thus had to exclude the possibility that the extrachromosomal arrays generated in our transformation experiments might mimic this effect rather than reflect the isolation of the *sdc-1* gene. Neither adjacent cosmids nor two clones, containing X chromosome sequences, previously shown to act as feminizing elements in transformation experiments[15], can rescue the *sdc-1* mutant phenotypes (Fig. 2).

To characterize transcripts derived from the *sdc-1* region, a 17-kb genomic clone containing *sdc-1* rescuing activity (pTY51) was subdivided into six fragments that were used individually to probe poly(A)$^+$ RNA isolated from a wild-type hermaphrodite strain and from a male-producing *him-8* strain[16] (not shown). We identified a single 3.8-kb transcript that spans most of the 9-kb *sdc-1* interval. Analysis of the developmental time course of the *sdc-1* transcript showed that the transcript is preferentially expressed during the embryonic period (Fig. 3). The *sdc-1* transcript levels decrease as development proceeds through the larval stages and then increase in adults (Fig. 3). This developmental expression pattern is consistent with temperature-shift experiments that demonstrate a requirement for *sdc-1* during the first half of embryogenesis[10]. It is also consistent with the *sdc-1* phenotype being maternally rescuable, indicating that *sdc-1* should be expressed in the adult hermaphrodite germ line[9].

DNA sequence analysis revealed that *sdc-1* encodes a 1,203-amino-acid protein containing seven zinc-finger motifs (Fig. 4*a*). Although five of the motifs in *sdc-1* resemble the consensus C_2H_2 TFIIIA motif[17,18] (Fig. 4*b*), two show more variability than those in most other proteins containing many zinc-fingers[17,19–21]. The TFIIIA zinc-finger DNA-binding motifs consist of conservatively positioned pairs of cysteine and histidine residues that coordinate a metal ion (usually zinc)[17,18]. The *sdc-1* zinc finger motifs that resemble the consensus TFIIIA motif are interspersed with those that have the histidine pair replaced with a cysteine–histidine pair. This type of variation is observed in some zinc-finger proteins known to bind DNA, including the major histocompatibility complex enhancer binding protein, MBP-1 (ref. 22), the SW15 protein involved in yeast mating-type switching[23] and the PRDII-BF1 protein that positively regulates the human interferon β gene[24]. In contrast to most multiple zinc-finger proteins[17,19–21], the *sdc-1* fingers are not arranged in a regularly spaced tandem array, but rather in four widely spaced clusters of single and paired motifs (Fig. 4*c*). In this regard *sdc-1* is reminiscent of MBP-1 and PRDII-BF1, which have structurally distinct zinc-fingers positioned in the amino- and carboxy-terminal regions of their protein products. In light of the similarity between *sdc-1* and known DNA binding proteins, it seems likely that *sdc-1* functions as a maternally supplied, embryonic transcription factor. However, we cannot exclude the interesting possibility of *sdc-1* acting primarily as an RNA-binding protein such as the p43 zinc-finger protein that preferentially binds 5S RNA in *Xenopus*[25].

Genetic evidence has already indicated that *sdc-1*, together with *sdc-2* and the newly identified *sdc-3* gene[26,27] (not shown in Fig. 1), promotes hermaphrodite sexual development by negatively regulating *her-1*, a sex determination gene required for male development[10,11]. Molecular confirmation has come from the observation that *her-1* transcript levels, normally very low in hermaphrodites but high in males[28,29], are greatly elevated in XX *sdc-1*, *sdc-2* (ref. 28) and *sdc-3* (L. DeLong, J. Plenefisch, R. Klein and B.J.M., manuscript in preparation) mutant animals. These data, together with the identification of an *sdc-1* zinc-

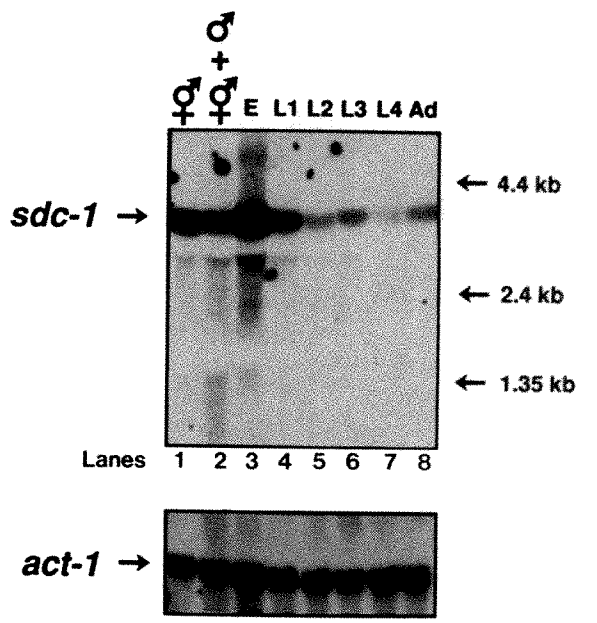

FIG. 3 Developmental regulation of *sdc-1* transcript levels. A northern blot of poly(A)$^+$ RNAs (10 μg per lane) from different developmental stages of the *C. elegans* life cycle. Lanes 1 and 2, RNA isolated from mixed-staged cultures of wild-type N2 strain (♂) or a male-producing *him-8* strain (♂ and ♂, approximately 30% males). Lanes 3–8, RNA isolated from synchronized hermaphrodite cultures grown to different developmental stages—eggs (E), the four larval stages (L1–L4), and adults before the onset of egg production (Ad). The northern blot was probed individually with a 3.0-kb *Eco*RI fragment from complementary DNA clone λTY1, and with an *act-1* specific actin clone (pW-16-210; gift of M. Krause) to control for the amount of mRNA in each lane. RNA size markers appear on the right of the autoradiograph. Mixed-stage RNA from the *him-8* strain does not reveal any alternative, male-specific transcript from the *sdc-1* region.

METHODS. Nematodes were grown in liquid culture and synchronized as described in ref. 36. Synchronized L1 larval populations were fed and L1 larvae collected after 2 h at 20 °C. L2 (26 h), L3 (34 h), L4 (44 h) and young adults (50 h) were collected at later times after confirming the staging by microscopic examination of animals using Nomarski optics. To isolate RNA, frozen packed nematodes (5 ml) were crushed under liquid nitrogen with a mortar and pestle, transferred to a tube containing 5 ml phenol, 5 ml $CHCl_3$ and 15 ml 1% SDS 1% β-mercaptoethanol, 10 mM EDTA, 0.2 M Tris-Cl, pH 7.5, and 0.5 M NaCl. The mixture was sonicated for 1 min in six bursts using a microtip at maximum power, and the phases separated by centrifugation. After four subsequent phenol extractions, the aqueous layer was loaded onto an oligo d(T) column, and poly(A)$^+$ RNA was isolated as described in ref. 37. Electrophoresis, blotting and probing of northern blots were as described in ref. 37.

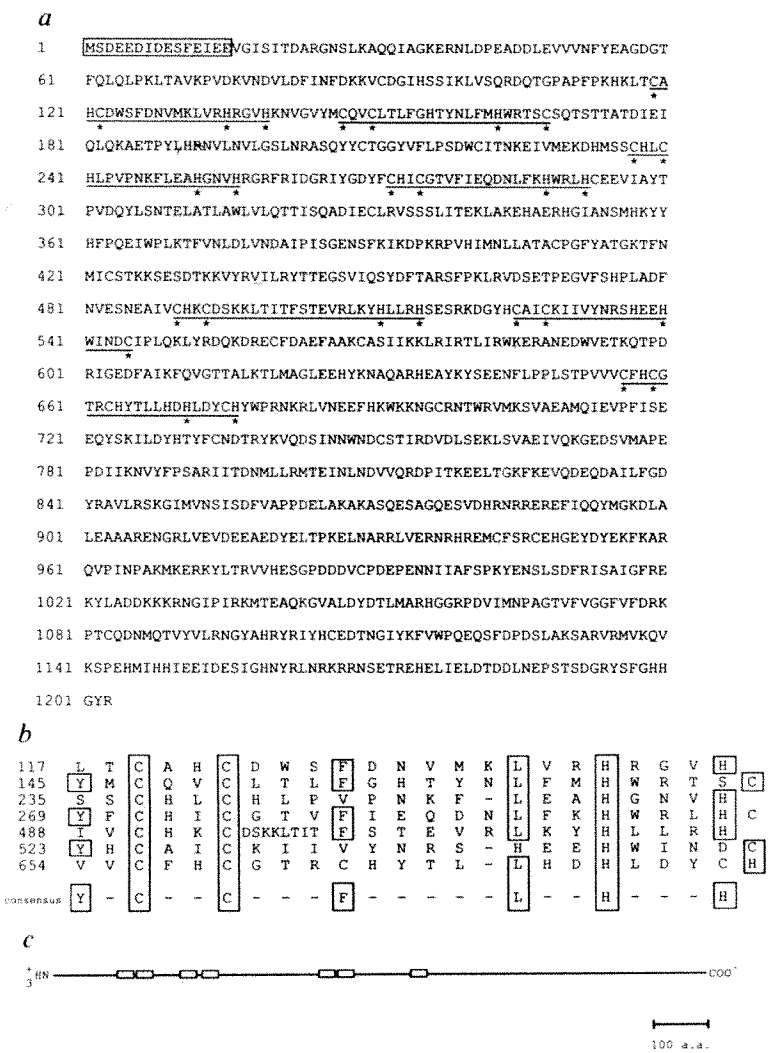

FIG. 4 *a*, The deduced amino-acid sequence of *sdc-1* (single-letter code). A highly acidic region of −9 charge is boxed. Each of the zinc fingers is underlined, and the relevant cysteines and histidines are indicated by stars. The GenBank-EMBL accession number for the *sdc-1* DNA sequence is X58520. *b*, The sequence of the seven zinc-finger motifs found in *sdc-1*. The numerical position of the initial amino acid of each zinc-finger is located to the left of the amino-acid sequences. The TFIIIA-like zinc-finger motif consensus sequence[17] is listed below; *sdc-1* amino acids matching the consensus are boxed. *c*, A schematic of the *sdc-1* protein highlighting (boxes) the position of the seven zinc-finger motifs within the protein.

METHODS. A mixture of two probes from the left and right side of pTY66 was used to isolate three independent *sdc-1* cDNA clones from a mixed stage hermaphrodite library (gift of S. Kim). The cDNAs (λTY1, 3.6 kb; λTY7, 2.8 kb; and λTY3, 2.2 kb) were shown by restriction analysis and partial sequence analysis to contain different sized cDNA clones of the same mature message. Further, we demonstrated that these three cDNAs hybridize only to genomic sequences corresponding to the *sdc-1* gene as defined by transgenic rescue. Because the largest cDNA was incomplete, the clone of the 5′ end of *sdc-1* message was isolated by polymerase chain reaction (PCR) techniques. The sequence of *sdc-1* message was compiled from sequencing of the λTY1 cDNA insert, genomic sequences 5′ of this cDNA, and 5′ end PCR products. In addition, genomic sequences corresponding to at least 90% of the cDNA sequences were determined and found to be identical. Many transcripts in *C. elegans* are *trans*-spliced to one of two leader RNAs[38]. We amplified by PCR and sequenced the 5′ end of the *sdc-1* message using an oligonucleotide of SL1 *trans*-splice leader (5′-TCTAGAATTCCGCGGTTTAATTACCCAAGTTTG-3′) and a complementary oligonucleotide (5′-AAATCCACTGGTTTCACAGCTG-3′) from the 5′ end of our largest cDNA clone. Mature *trans*-spliced message is 3.8 kb without the poly(A) tail. But primer extension of poly(A)$^+$ egg RNA using an oligonucleotide 54 bp 3′ of the *trans*-splicing site (5′-CCTCTTCGATTTCAAAGCTTTCATC-3′) suggests that most (>90%) of egg *sdc-1* RNA is not *trans*-spliced and probably initiates in a cluster 70–80 bp upstream of the *trans*-splicing site.

finger motif, suggest that a component of the early response to the X/A ratio involves transcriptional regulation. But the sexual transformation of XX animals towards the male fate is not as complete in *sdc-1* mutants as it is in *sdc-2* or *sdc-3* mutants, suggesting that *sdc-1* does not act alone in regulating the downstream sex determination genes[7]. A similar situation arises in the control of dosage compensation. Ultimately, *sdc-1* in combination with other *sdc* genes must activate the downstream hermaphrodite-specific genes that equalize X-chromosome expression between the sexes.

If *sdc-1* is a transcriptional regulator, then its target(s) might either be other *sdc* genes or the downstream genes that specifically control sex determination or dosage compensation. In the latter case, *sdc-1* would function simultaneously as a negative regulator of sex determination genes and a positive activator of dosage compensation genes. In this regard, the *sdc-1* protein might be similar to the glucocorticoid receptor[30] or the yeast MCM1 protein. The MCM1 protein interacts with the yeast mating-type protein MATα1 to activate transcription of α-specific genes in α cells[31], but interacts with the yeast mating-type protein MATα2 to repress transcription of **a**-specific genes in α cells[32]. Delineating the *sdc-1* targets and determining the functional roles of the zinc fingers will greatly improve our understanding of the mechanisms underlying these complex developmental decisions. □

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Cloning of a complementary DNA for a protein-tyrosine kinase that specifically phosphorylates a negative regulatory site of p60$^{c\text{-}src}$

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THE protein-tyrosine kinase activity of the proto-oncogene product p60$^{c\text{-}src}$ is negatively regulated by the phosphorylation of a tyrosine residue close to the C terminus, tyrosine 527 (refs 1–11). The phosphorylation might be catalysed by a so-far-unidentified tyrosine kinase, distinct from p60$^{c\text{-}src}$ (ref. 7). Recently we purified a protein-tyrosine kinase that specifically phosphorylates tyrosine 527 of p60$^{c\text{-}src}$ from neonatal rat brain[8,12,13]. We have now confirmed the specificity of this enzyme by using a mutant p60$^{c\text{-}src}$ that has a phenylalanine instead of tyrosine 527, and cloned a complementary DNA that encodes the enzyme. The enzyme is similar to kinases of the *src* family in that it has two conserved regions, Src-homology regions 2 and 3, upstream of a tyrosine kinase domain. The amino-acid identity of each region is no more than 47%, however, and the enzyme lacks phosphorylation sites corresponding to tyrosines 416 and 527 of p60$^{c\text{-}src}$ and has no myristylation signal. These results suggest that this protein-tyrosine kinase, which might negatively regulate p60$^{c\text{-}src}$, represents a new type of tyrosine kinase.

The specificity of our novel tyrosine kinase (termed 'c-Src kinase' or 'CSK') was further confirmed by an experiment using kinase-inactive mutants of p60$^{c\text{-}src}$. Wild-type p60$^{c\text{-}src}$ (p60^{WT}) and two kinds of mutants (p60^{R295} and p60^{R295F527}) were expressed in yeast cells[11,14]. Mutant p60^{R295} was generated by introducing a mutation into the codon for lysine 295 so that it specified arginine; p60^{R295F527} was generated from p60^{R295} by introducing a further mutation in the codon for Tyr 527 so that it specified phenylalanine. When synthesized in yeast, p60^{WT} is phosphorylated to low stoichiometry at Tyr 527 only[14], and p60^{R295} is not phosphorylated at all[11], so these molecules should be substrates for kinases that are specific for Tyr 527. These p60$^{c\text{-}src}$ derivatives were purified from yeast cell lysates with a monoclonal antibody, which does not interfere with phosphorylation by CSK. Immunoprecipitates containing similar quantities of the three proteins (Fig. 1*b*) were incubated with highly purified CSK (Fig. 1*a*). The phosphorylation of p60^{WT} was greatly increased by the addition of CSK (Fig. 1*c*, lanes 1 and 2). Mutant p60^{R295} was also phosphorylated (lanes 3 and 4), whereas p60^{R295F527} was not phosphorylated at all (lanes 5 and 6). These results imply that CSK is highly specific for Tyr 527, and show that CSK does not redirect the autophosphorylation activity of p60$^{c\text{-}src}$ to Tyr 527. When these p60$^{c\text{-}src}$ mutants were incubated with purified insulin receptor, a tyrosine kinase with broad substrate specificity, neither p60^{R295} nor p60^{R295F527} was markedly phosphorylated (data not shown). As specific phosphorylation of Tyr 527 of p60$^{c\text{-}src}$ by any other kinase has not been reported, CSK is a strong candidate for the specific kinase involved in the regulation of p60$^{c\text{-}src}$.

To characterize the enzyme further, we cloned the cDNA that encodes CSK and deduced its primary structure (Fig. 2). The complete nucleotide sequence of the cDNA is shown in Fig. 2. The cDNA insert has a single open reading frame which encodes a protein of 450 amino-acid residues with a relative molecular mass of 50,753.

To show that the cDNA clone encodes CSK, we transfected the cDNA into yeast cells carrying the mutant p60$^{c\text{-}src}$ proteins (Fig. 3*a*), and examined whether the cDNA product could

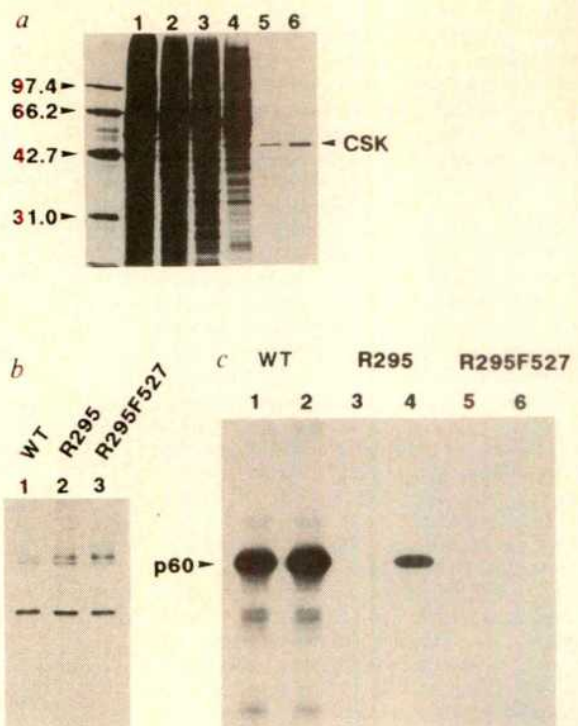

FIG. 1 Phosphorylation of mutant p60$^{c\text{-}src}$ by CSK. *a*, Sample of each purification step of CSK was analysed by SDS–PAGE and staining with silver. Lane 1, Nonidet P-40 extract; lane 2, DEAE–cellulose column chromatography; lane 3, poly(Glu, Tyr) Sepharose CL-4B; lane 4, Mono Q; lane 5, Sephacryl S200HR; lane 6, Mono S. Positions of relative molecular mass (M_r) markers (in $M_r \times 10^{-3}$) are indicated. *b*, Expression of p60^{WT} (lane 1), p60^{R295} (lane 2) and p60^{R295F527} (lane 3) in yeast cells was detected by immunoblotting of the immunoprecipitates. The bands above p60 are immunoglobulin. *c*, Phosphorylaion of p60$^{c\text{-}src}$ by CSK was determined by incubating immunoprecipitates of p60^{WT} (lanes 1 and 2), p60^{R295} (lanes 3 and 4) and p60^{R295F527} (lanes 5 and 6) with (lanes 2, 4 and 6) or without (lanes 1, 3 and 5) CSK. The reaction products were analysed by SDS-PAGE and autoradiography.

METHODS. *a*, CSK was purified from the membrane fraction of neonatal rat brain by sequential column chromatography. The method was essentially the same as that described previously[8], but with changes to improve the yield and purity. In brief, after DEAE-cellulose column chromatography, the sample was applied to a poly(Glu, Tyr) Sepharose CL-4B column, and eluted with 0.1 M NaCl. The eluate was applied to a Mono Q column, and eluted with a linear gradient of 0–0.35 M NaCl. The materials eluted at ~0.25 M NaCl were separated on a Sephacryl S200HR column. Finally, the active fractions were applied to a Mono S column, and eluted with a linear gradient of 0–0.2 M NaCl. CSK was eluted at about 0.1 M NaCl. By this improved method, we obtained about 100 μg highly purified CSK from 300 g neonatal rat brains. *b*, Yeast cells expressing p60^{WT} (pMX), p60^{R295} (JC851) and p60^{R295F527} (JC861) were generated as described previously[11,13]. Cell culture, lysis, immunoprecipitation with monoclonal antibody 327 and immunoblotting with the same antibody were carried out by the method of Cooper and Runge[14], except that the antigens were detected with peroxidase–antiperoxidase complex. In this experiment, immunoprecipitates obtained from 1×10^9 yeast cells were analysed. *c*, Phosphorylation by CSK was determined as described previously[8]. The reaction mixture (10 μl) contained 50 mM Tris–HCl, pH 7.4, 3 mM $MnCl_2$, 0.1 mM Na_3VO_4, 1 μM [γ-^{32}P]ATP (74 kBq), immunoprecipitate of p60$^{c\text{-}src}$ obtained from 1×10^9 yeast cells and 50 ng of CSK. After incubation for 10 min at 30°C, the reaction was terminated by adding 10 μl of SDS sample buffer and boiling for 2 min. The sample was then subjected to SDS-PAGE, and the phosphoproteins were located by autoradiography.

phosphorylate Tyr 527 of $p60^{c\text{-}src}$. Expression of the cDNA was confirmed by immunoblotting with antibody raised against bacterially expressed protein (Fig. 3*c*). The antibody specifically reacted with brain CSK and precipitated CSK (data not shown), indicating that the cDNA product was CSK. The degree to which $p60^{c\text{-}src}$ was phosphorylated in yeast cells was estimated by immunoblotting with anti-phosphotyrosine antibody. Autophosphorylated $p60^{WT}$ and its supposed substrates were detected (Fig. 3*b*, lane 1). When the cDNA was coexpressed with $p60^{R295}$, tyrosine phosphorylation of a band corresponding to $p60^{R295}$ was detected (lane 3), whereas, for coexpression with $p60^{R295F527}$, tyrosine phosphorylation was not detected (lane 5). These results are consistent with those of experiments *in vitro* (Fig. 1*c*), and indicate that the cDNA product specifically phosphorylates Tyr 527 of $p60^{c\text{-}src}$ even in intact yeast cells. Thus the cDNA clone encodes CSK, which seems to regulate $p60^{c\text{-}src}$ under physiological conditions.

The sequence of CSK has all the features of a protein-tyrosine kinase[15], having the highest similarity to *src*-family kinases. Among them, $p60^{c\text{-}src}$ (Src)[16] is the most similar, but with only 46% amino-acid identity. Also, all Src-related kinases have the sequence DLRAAN (single-letter amino-acid code) in subdomain VI, rather than the DLAARN of CSK and other kinases outside the *src* family. Other protein-tyrosine kinases, including receptor-type tyrosine kinases[17–20], are similar to CSK in the kinase domain, with sequence identities ranging from 39 to 46% (Fig. 4*a*).

CSK lacks a tyrosine residue at a site corresponding to tyrosine 416 of $p60^{c\text{-}src}$. Tyr 416 is a site for autophosphorylation in $p60^{c\text{-}src}$. The members of the *src* family share a highly conserved sequence around this site, and are autophosphorylated at a tyrosine residue equivalent to Tyr 416. CSK, however, shows no autophosphorylation (Fig. 1*c*), a deficiency that may be important for its substrate specificity or regulation.

The members of the *src* family also share a highly conserved C-terminal sequence which includes a tyrosine residue equivalent to Tyr 527. This tyrosine residue could be phosphorylated and serve as a site for negative regulation[21]. By contrast, CSK has a stop codon at the site corresponding to Tyr 527. Thus, on the basis of its homology with other protein-tyrosine kinases and its lack of autophosphorylation, CSK would seem to be a member of a new family of protein-tyrosine kinases.

Upstream of the kinase domain, CSK contains the 'Src-homology regions' 2, 2′ and 3 (SH2, SH2′ and SH3 regions; Fig. 4*b*). These exist in cytoplasmic protein-tyrosine kinases[22], where they are important for function[23], and in a viral oncoprotein, $p47^{gag\text{-}crk}$ (ref. 24), phospholipase C-γ (PLC)[25] and GTPase activator protein (GAP)[26]. Because $p47^{gag\text{-}crk}$ regulates the activity of cellular tyrosine kinases, and both PLC[27] and GAP[28] can be phosphorylated on tyrosine, SH2 and SH3 could be

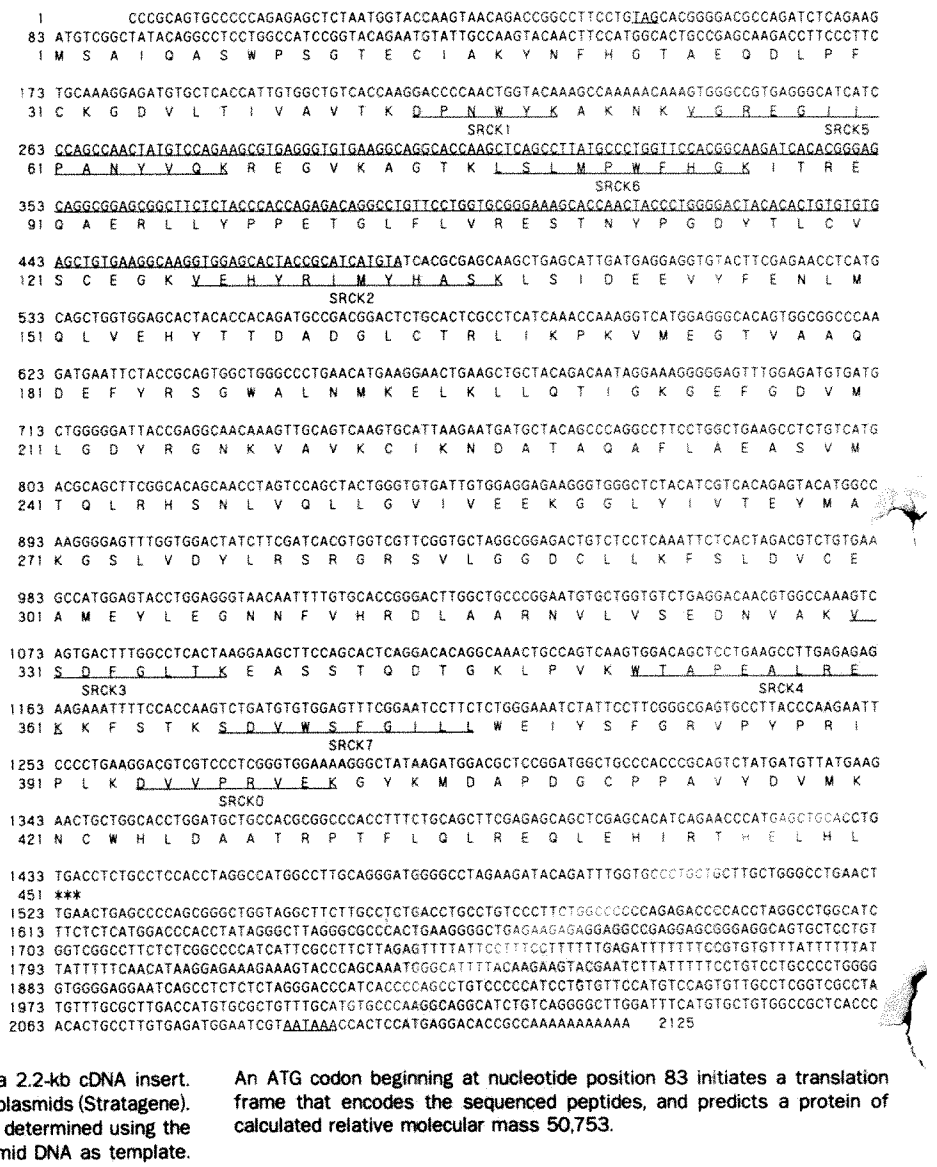

FIG. 2 Nucleotide sequence and deduced amino-acid sequence (single-letter code) of CSK. The 2,125-nucleotide cDNA insert contains a single open reading frame that encodes a protein of 450 amino acids. An in-frame stop codon in the 5′-untranslated region and a polyadenylation consensus sequence in the 3′-untranslated region are underlined. Amino-acid sequences of eight peptides obtained by lysyl-endopeptidase digestion of CSK are indicated by underlines designated as SRCK0-7. A DNA fragment amplified by the polymerase chain reaction (PCR) and used as a probe to screen a cDNA library is indicated by an underline.

METHODS. CSK was highly purified from neonatal rat brains as described in the legend to Fig. 1. The enzyme (50 μg) was digested with lysyl-endopeptidase. The resulting peptides were separated by HPLC and subjected to automated gas phase microsequencing. Four sequences (DPNWYK in SRCK1, VECYRIMY in SRCK2, KWTAPEA in SRCK4 and PANYVGK in SRCK5) were used to design degenerate oligonucleotide primers for PCR. The first strand of cDNA, which was used as a template for PCR, was synthesized using antisense primer of SRCK4 with poly(A)$^+$ RNA template prepared from neonatal rat brains. A PCR between-sense primer of SRCK5 and antisense primer of SRCK2 yielded an amplified product of 240 bp (underlined in the figure). This product was used as a probe to screen a λgt10 cDNA library. The cDNA library was constructed as follows: poly(A)$^+$ RNA obtained from neonatal rat brains (postnatal day 2–4) was reverse-transcribed with an oligo(dT) primer. Double-stranded cDNA was synthesized using a cDNA synthesis system (Amersham), and size-fractionated (>1 kb). The cDNA was ligated into λgt10 vector, and packaged *in vitro* using Gigapack Gold (Stratagene). Part of the recombinants obtained was amplified, and the sub-library was used for screening. The probe was labelled using Multiprime DNA labelling system (Amersham), and the hybridization was carried out at high stringency. A single positive clone was gained from 450,000 recombinants, and was shown to contain a 2.2-kb cDNA insert. The cDNA insert was subcloned into Bluescriptll SK+ plasmids (Stratagene). The complete nucleotide sequence of the cDNA was determined using the Sequenase system (USB) with double-stranded plasmid DNA as template. An ATG codon beginning at nucleotide position 83 initiates a translation frame that encodes the sequenced peptides, and predicts a protein of calculated relative molecular mass 50,753.

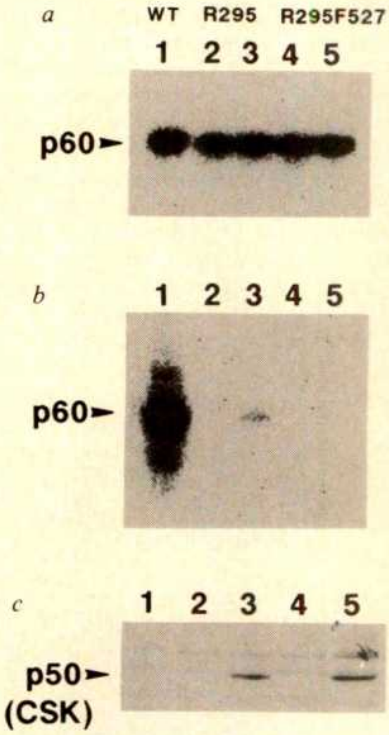

FIG. 3 Phosphorylation of Tyr 527 by the cDNA product in yeast cells. The cDNA was transfected into yeast cells carrying the mutant $p60^{c\text{-}src}$ proteins, and their tyrosine phosphorylation was estimated by immunoblot analysis with anti-phosphotyrosine antibody. *a*, Expression of $p60^{c\text{-}src}$ in yeast cells carrying $p60^{WT}$ (lane 1), $p60^{R295}$ (lane 2), $p60^{R295}$ and pHMCSK (lane 3), $p60^{R295F527}$ (lane 4), and $p60^{R295F527}$ and pHMCSK (lane 5) was confirmed by immunoblotting with monoclonal antibody 327. *b*, Phosphorylation of $p60^{c\text{-}src}$ in the same cultures was detected by immunoblotting with anti-phosphotyrosine antibody. *c*, Expression of the CSK cDNA product (lanes 3 and 5, p50) was detected by immunoblotting with an antibody raised against bacterially expressed protein.

METHODS. A fragment containing the open reading frame (nucleotides 71–1,452, Fig. 2) was amplified by PCR using two primers attached to *Sal*I linkers, then the *Bgl*II–*Xho*I fragment (71–1,405) was exchanged for the same fragment of original clone to exclude the possibility of mutations occurring during PCR. The residual fragment (1,406–1,452) was confirmed not to contain any mutations by sequencing the fragment. The *Bgl*II–*Sal*I fragment obtained was ligated to *Bam*HI–*Sal*I fragment of pHM209, a derivative of pHM153[32] containing the *TRP1* gene. The expression plasmid (pHMCSK) was transfected into the yeast strain expressing $p60^{R295}$ (JC851) and $p60^{R295F527}$ (JC861). Trp^+ and Ura^+ transformants were selected, and grown as described previously[11,13]. Yeast cells expressing $p60^{WT}$ (lane 1), $p60^{R295}$ (lane 2), $p60^{R295}$ and pHMCSK (lane 3), $p60^{R295F527}$ (lane 4), and $p60^{R295F527}$ and pHMCSK (lane 5) were collected, and immediately lysed in SDS-PAGE sample buffer by boiling. Each sample (equivalent to 2×10^8 cells) was then immunoblotted. Detection of $p60^{c\text{-}src}$ was with monoclonal antibody 327 (*a*), and phosphotyrosine was detected with monoclonal antibody PY20 (ICN) (*b*). In this experiment, these monoclonal antibodies were visualized with ^{125}I-labelled anti-mouse IgG (sheep). The radioactivity bound was measured using BioImage-Analyzer BAS2000 (Fuji Film). Expression of the CSK cDNA product was detected with antibody raised against bacterially expressed protein (*c*). Bluescriptll SK+ containing the *Bgl*II–*Sal*I fragment was digested with *Xba*I, filled with Klenow fragment, and ligated to allow in-frame translation. The expression plasmid was transfected into *Escherichia coli* (TG1). The lacZ fusion protein was induced by IPTG, and purified from inclusion bodies by preparative SDS-PAGE. Antiserum was obtained from a rabbit immunized with the purified protein. The rabbit polyclonal antibody was detected with peroxidase–antiperoxidase complex.

a

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                 ---------- I ----------          ------ II -------  ----- III -----
CSK (193)  KELKLLQTIGKGEFGDVMLGDYRG----------NKVAVKCIKNDA-----TAQAFLAEASVMTQL-
SRC (265)  ESLRLEVKLGQGCFGEVWMGTWNG---------TTRVAIKTLKPGNM----SPEAFLQEAQVMKKL-
ABL (239)  TDITMKHKLGGGQYGEVYEGVWKK---------YSLTVAVKTLKEDTM----EVEEFLKEAAVMKEI-
DER (808)  AELRKGVVLGMGAFGRVYKGVWVPEGENV----KIPVAIKELLKSTGAE--SSEEFLREAYIMASE-
RET (455)  KNLVLGKILGEGEFGKVVKATAFH---------LKTVAVKMLKENASPS--ELRDLLSEFNVLKQV-
ROS (154)  EKLTLRLLLGSGAFGEVYEGTAVDILGVGSG--EIKVAVKTLKKGSTDQ--EKIEFLKEAHLMSKF-

                 ----- IV ------   ----------- V ------------
-RHSNLVQLLGVIVEEK--GGLYIVTEYMAKGSLVDYLRSRGRSV------------------------
-RHEKLVQLYAVVSE----EPIYIVTEYMSKGSLLDFLKGEMGKY------------------------
-KHPNLVQLLGVCTRE---PPFYIITEFMTYGNLLDYLRECNRQE------------------------
-EHVNLLKLLAVCMS----SQMMLITQLMPLGCLLDYVRNNRDK-------------------------
-NHPHVIKLYGACSQD---GPLLLIVEYAKYGSLRGFLRESRKVGPGYLGSGGSRNSSSLDHPDERA-
-NHPNILKQLGVCLLN---EPQYIILELMEGGDLLTYLRKARMATFYGPL-------------------

                 ---------- VI ---------------------  --------- VII ----¶---
-LGGDCLLKFSLDVCEAMEYLEGNNFVHRDLAARNVLVSEDN--------VAKVSDFGLTKEASSTQ------
-LRLPQLVDMAAQIASGMAYVERMNYVHRDLRAANILVGENL--------VCKVADFGLARLIEDNEYTAR--
-VNAVVLLYMATQISSAMEYLEKKNFIHRDLAARNCLVGENH--------LVKVADFGLSRLMTGDTYTAH--
-IGSKALLNWSTQIAKGMSYLEEKRLVHRDLAARNVLVRLL---------AGEDHDFGLAKLLSSDSNEYKA-
-LTMGDLISFAWQISQGMQYLAEMKLVHRDLAARNILVAEGR--------KMKISDFGLSRDVYEEDSYVKR-
-LTLVDLVDLCVDISKGCVYLERMHFIHRDLAARNCLVSVKDYTSPR--IVKIGDFGLARDIYKNDYYRKR-

                 ---- VIII -----  ----------------- IX ------------
-DTGKLPVKWTAPEAL--REKKFSTKSDVWSFGILLWEIYSFGRVPYPR-
-QGAKFPIKWTAPEAA--LYGRFTIKSDVWSFGILLTELTTKGRVPYPG-
-AGAKFPIKWTAPESL--AYNKFSIKSDVWAFGVLLWEIATYGMSPYPG-
-AGGKMPIKWLALECI--HNRVFTSKSDVWAFGVTIWELLTFGGQPHEN-
-SQGRIPVKWMAIESL--FDHIYTTQSDVWSFGVLLWEIVTLGGNPYPG-
-GEGLLPVRWMAPESL--MDGIFTIQSDVWSFGILIWEILTLGHQPYPA-

                 ---------- X ----------  ------------ XI --------------  ¶
-IPLKDVVPRVEKGYKMDAPDG--CPPAVYDVMKNCWHLDAATRPTFLQLREQL--EHIRTHELHL*
-MVNREVLDQVERGYRMPCPPE--CPESLHDLMCQCWRRDPEERPTFEYLQAFL--EDVFTSTEPQYQP
-IDRSQVYELLEKDYRMKRPEG--CPEKVYELMRACWQWNPSDRPSFAEIHQAF--ETMFQESSISDEV
-IPAKDIPDLIEVGLKLEGPEI--CSLDIYCTLLSCWHLDAAMRPTFKQLTTVF--AEFARDPGRYLAI
-IPPERLFNLLKTGHRMERPDN--CSEEMYRLMLQCWKQEPDKRPVFADISKDL--EKMMVKRRDYLDL
-HSNLDVLNYVQTGGRLEPPRN--CPDDLWNLMTQCWAQEPDQRPTFHRIQDQL--QLFRNFFLNSIYK
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b

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                 ---------------------- SH3 ------------------------
CSK  (14)  CIAKYNFHGTAEQDLPFCKGDVLTIVAVTKDPNWYKAKNKVGREGIIPANYVQK
SRC  (86)  FVALYDYESRTETDLSFKKGERLQIVNNTEGDWWLAHSLTTGQTGYIPSNYVAP
CRK (373)  VRALFDFKGNDDGDLPFKKGDILKIRDKPEEQWWNAEDMD-GKRGMIPVPYVEK
PLC (795)  VKALFDYKAQREDELTFTKSAIIQNVEKQEGGWWRGDYGG-KKQLWFPSNYVEE
GAP (282)  RAILPYTKVPDTDEISFLKGDMFIVHNELEDGWMWVTNLRTDEQGLIVEDLVEE

                 ------------------------ SH2 -------------------------
CSK  (82)  WFHGKITREQAERLLVPPET--GLFLVRESTNVPGDYTLCVSCEG-----KVEHYR
SRC (148)  WYFGKITRRESERLLLNPENPRGTFLVRESETTKGAYCLSVSDFDNAKGLNVKHYK
CRK (248)  WYWGRLSRGDAVSLLQGQRH--GTFLVRDSGSIPGDFVLSVSESS-----RVSHYI
PLC (668)  WYHASLTRAQAEHMLMRVPRD-GAFLVRKRNE-PNSYAISFRAEG-----KIKHCR
GAP (178)  WYHGKLDRTIAEERLRQAGKS-GSYLIREDSRRPGSFVLSFLSQTN----VVNHFR

                                                  --- SH2' ---
-----------------IMYHA-SKLSIDEEVYFENLMQLVEHY
-----------------IRKLDSGGFYITSRTQFSSLQQLVAYY
VNSLGPAGGRRAGGEGPGAPGLNPTRFLIGDQVEDSLPSLLEFY
-------------------VQGEGQTVMLGNSEFDSLVDLISYY
-----------------IIAHCGDYYIGG-RRESSLSDLIGYY
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FIG. 4 *a*, Amino-acid alignment of the conserved kinase domain in CSK; c-*src* gene product (SRC; a representative of the *src* subfamily)[16]; c-*abl* gene product (ABL, *abl* subfamily)[17]; *Drosophila* gene product related to the EGF receptor (DER, EGF receptor subfamily)[18]; cellular oncogene product activated by recombination (RET, PDGF receptor subfamily)[19] and c-*ros* gene product (ROS; insulin receptor subfamily)[20]. Underlined bold type indicates residues identical to the kinase domain sequence in CSK. Roman numbers designate subdomains conserved in protein kinases[15]. The number in parentheses is the first amino acid of sequence shown. Sequence identities are 46% (SRC), 46% (ABL), 41% (DER), 39% (RET) and 41% (ROS). Sequence identities to other *src*-family kinases are 45% (*fgr*), 44% (*lyn*), 43% (*yes*), 43% (*hck*), 42% (*fyn*) and 42% (*lck*)[15]. The percentage identities were determined over the length of the kinase domain of CSK; residues in insertions were ignored for these calculations. Arrows denote the residues corresponding to Tyr 416 and Tyr 527 of $p60^{c\text{-}src}$. *b*, Amino-acid alignment of SH2, SH2′ and SH3 regions in CSK, c-*src* gene product (SRC)[16], *gag-crk* gene product (CRK)[24], phospholipase C-γ (PLC)[25] and GTPase activator protein (GAP)[26]. Total sequence identities in these regions are 47% (SRC), 40% (CRK), 30% (PLC) and 27% (GAP). The percentage identities were determined as described above.

important in the regulation of tyrosine kinase and in the interaction with cellular proteins that serve as substrates or regulators. These regions in CSK could therefore be involved in recognition of its substrate, $p60^{c\text{-}src}$, or interaction with a regulator that modulates its kinase activity.

CSK lacks a myristylation signal at the N terminus and has no potential membrane-spanning sequences, suggesting that it is a cytosolic protein. Although CSK was originally purified from membrane fractions, immunoblotting with anti-CSK antibody indicates that CSK is also present in the soluble fraction (data not shown). This is consistent with a soluble mutant of $p60^{c\text{-}src}$ being also phosphorylated effectively at Tyr 527[29]. CSK recovered from membrane fractions might have been bound to $p60^{c\text{-}src}$.

Northern blot analysis using a probe for CSK mRNA identified a 2.4-kilobase (kb) transcript which was present in all adult rat tissues examined, but markedly concentrated in neonatal brain (data not shown). Because $p60^{c\text{-}src}$ is concentrated in developing rat brain[30], CSK could have an important role in the regulation of $p60^{c\text{-}src}$ during neuronal differentiation. In thymus and spleen, cross-hybridizing transcripts of 2.2 kb were detected at a similar level to that of the neonatal transcript. This raises the possibility that a subtype of CSK exists in these tissues, which indeed contain different types of *src*-family kinases, such as the gene products of *lck*, *fyn* and *lyn*. These kinases might be involved in the regulation of lymphoid-cell differentiation[31]. Therefore, distinct subtypes of CSK may regulate *src*-family kinases in some non-neuronal cell types. □

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Specific binding of antigenic peptides to cell-associated MHC class I molecules

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T LYMPHOCYTES recognize antigen in the form of peptides that associate with specific alleles of class I or class II major histocompatibility (MHC) molecules[1,2]. By contrast with the clear MHC allele-specific binding of peptides to purified class II molecules[3–6] purified solubilized class I molecules either bind relatively poorly[7] or show degenerate specificity[8–11]. Using photoaffinity labelling, we demonstrate here the specific interaction of peptides with cell-associated MHC class I molecules and show that this involves metabolically active processes.

The relative efficiency of the interaction of different peptides with the K^d molecule was assessed in a functional competition assay[12,13] using a K^d-restricted cytotoxic T lymphocyte (CTL) clone (Fig. 1*a*). Of the peptides recognized by K^d-restricted CTL, one of the most potent competitors was an octapeptide from the mouse malaria *P. berghei* circumsporozoite protein, CS P.b. 253–260 (ref. 14, and P.R. *et al.*, manuscript submitted) (Fig. 1*a*). The K^d-restricted peptide P198^{-}.14–24 (ref. 15) and a polyproline-containing peptide analogue (AYP_5TLA) (ref. 16) also competed, but the L^d-restricted peptides P91A^{-}. 12–24 (ref. 17) and LCMV NP 118–132 (ref. 18), and the D^b-restricted peptide Ad5 E1a 234–243 (ref. 19) failed to compete efficiently. The CS P.b. peptide was still an efficient competitor after N-terminal conjugation with the photoreactive iodo-4-azidosalicyloyl (IASA) group (Fig. 1*b*); the IASA group itself was not responsible for the competitive activity of the conjugates because the derivative having a deletion at Tyr 253 (IASA conjugated with the sequence IPSAEKI (one-letter amino-acid code) was less active by at least 100-fold compared with IASA-YIPSAEKI (Fig. 1*b*). Moreover, the IASA-YIPSAEKI derivative was recognized by some CS-specific CTL clones (not shown). Efficient competitor activity, but not recognition, was retained after biotinylation of the Lys 259 residue (Fig. 1*b*).

P815 ($H\text{-}2^d$) cells were incubated for 6 h at 37 °C with the [^{125}I]IASA-YIPSAEK(biotin)I probe and then photoactivated. Analysis of the total cell lysate by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) revealed a major labelled component with an apparent relative molecular mass of 45,000 (M_r 45K) and a minor one of 68K (Fig. 2, lane 1). The latter could be eliminated by preclearing with a BSA-specific monoclonal antibody (data not shown). The 45K labelled material in the lysate could be recovered nearly quantitatively by immunoprecipitation with a K^d-specific antibody, but not with an antibody specific for D^d or L^d (lanes 2–4). Similar results were obtained with the [^{125}I]IASA-YIPSAEKI probe (not shown). The 45K species was also detected by direct SDS-PAGE analysis in lysates from L cells ($H\text{-}2^k$) transfected with the K^d gene, but not in controls from transfectants expressing L^d or D^d (lanes 5–7). Furthermore, labelling was not significant in lysates of EL-4 or CH-27 cells expressing the class I molecules D^b and K^b, or D^k and K^k, respectively (lanes 8 and 9).

Photoaffinity labelling of K^d molecules on P815 cells with [^{125}I]IASA-YIPSAEK(biotin)I was completely inhibited by the unmodified CS P.b. peptide and by other peptides presented by K^d, but was not inhibited by D^b or L^d restricted peptides (Fig. 3). These results are in agreement with those obtained in the functional competition assay (Fig. 1) and with the MHC restriction patterns described for these peptides[14–19].

The specificity of MHC-peptide interactions has been investigated in a solid-phase binding assay using solubilized human

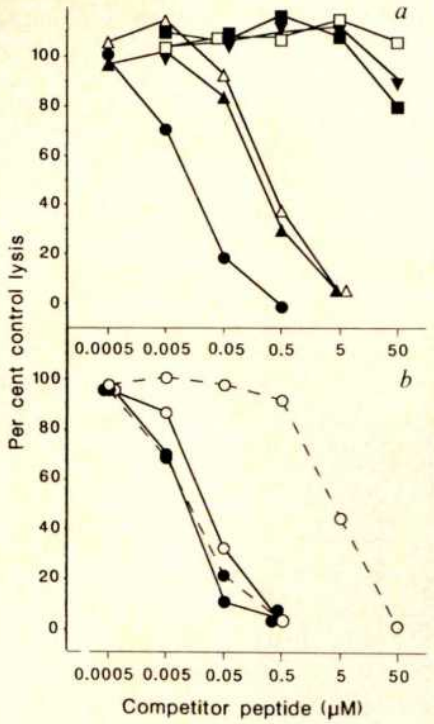

FIG. 1 Comparison of various antigenic peptides and photoreactive peptide conjugates as competitors in a functional competition assay. Peptide CS P.b. 253–260 (●—), P198⁻.14–24 (▲—), AYP$_5$TLA (Δ—), P91A⁻.12–24 (■—), LCMV NP 118–132 (□—), Ad5 E1a 234–243 (▼—) and photoreactive conjugates IASA–YIPSAEKI (○—), IASA–YIPSAEK(biotin)I (●– –), and IASA–IPSAEKI (○– –) were incubated with ^{51}Cr-labelled P815 target cells at the indicated (final) concentrations, together with the antigenic peptide CW3 170–182 (0.1 μM) and CTL–CW3/1.1 at a 5:1 CTL to target ratio.
METHODS. Peptides were synthesized on a RaMPS peptide synthesizer (Du Pont) using the (9-fluorenylmethoxycarbonyl) (Fmoc) strategy and purified on C-18 reverse-phase HPLC (Waters, Milford, MA). YIPSAEK(biotin)I was prepared by reacting Fmoc–YIPSAEKI with *N*-hydroxysuccinimidyl biotin (biotin–ONSu in dimethylsulphoxide in the presence of 1-hydroxybenzotriazole. The reaction mixture was treated with piperidine and the resulting YIPSAEK(biotin)I was purified by C-18 HPLC. The photoreactive derivatives were prepared essentially as described[29,30]. Freshly iodinated IASA–ONSu was reacted with YIPSAEK(biotin)I and the resulting IASA–YIPSAEK(biotin)I was purified by C-18 HPLC. To prepare the IASA–YIPSAEKI derivative, IASA–ONSu was reacted with the fully protected (except for the N terminus) CS P.b. peptide on the solid-phase resin and the product was then cleaved from the resin and purified by C-18 HPLC. All procedures involving photoreactive reagents were performed under dimmed light. Details of the competition assay are described in ref. 13. The ^{51}Cr-release assay was terminated after a 4-h incubation at 37 °C. The per cent control lysis was calculated as 100 × (% lysis with competitor minus background lysis) divided by (% lysis without competitor minus background lysis). Background lysis was less than 10%, and lysis without competitor was 54% and 63%, respectively, in *a* and *b*.

class I (HLA) molecules and peptides immobilized onto plastic[8–11]. Although this showed that class I molecules can indeed bind to antigenic peptides, the specificity of binding for many peptides was degenerate. Our experiments, which are based on photoaffinity labelling of class I molecules on living cells, establish that binding of an antigenic peptide to class I molecules can be highly restricted (Figs 1–3). These findings are in agreement with those demonstrating that endogenously processed peptides display a high degree of MHC allele-specificity[20–22].

As shown in Fig. 4, the photoaffinity labelling of K^d on living P815 cells at 37 °C increased steadily with the incubation time, whereas only weak labelling was observed at 4 °C. A similar reduction in labelling was observed at 37 °C in the presence of sodium azide and 2-deoxyglucose, the protein synthesis inhibitor cycloheximide, or brefeldin A, an inhibitor of protein transport from the endoplasmic reticulum[23] (Fig. 4*c*, *d* and *e*). Reduction in labelling was not due to a depletion of K^d molecules on the cell surface because flow cytometric analysis confirmed that cell-surface K^d expression was not substantially decreased for at least 6 h under the different incubation conditions (data not shown). Thus the accumulated labelling of K^d molecules on living P815 cells at 37 °C cannot simply be accounted for by direct binding of the photoprobe to the K^d cell-surface molecules available at the start of the incubation. As there was weak labelling in P815 cell lysates at 37 °C (Fig. 4*f*) and at 4 °C (data not shown), it is unlikely that this binding involves only intracellular K^d molecules available at the beginning of the incubation. The accumulated binding of exogeneous peptides to cell-associated class I molecules therefore seems to depend on metabolically active processes. These results are compatible with the interpretation that the peptide photoprobe binds predominantly to newly synthesized class I molecules. As already suggested[24–27], exogenous peptides might bind to 'nascent' class I heavy chains

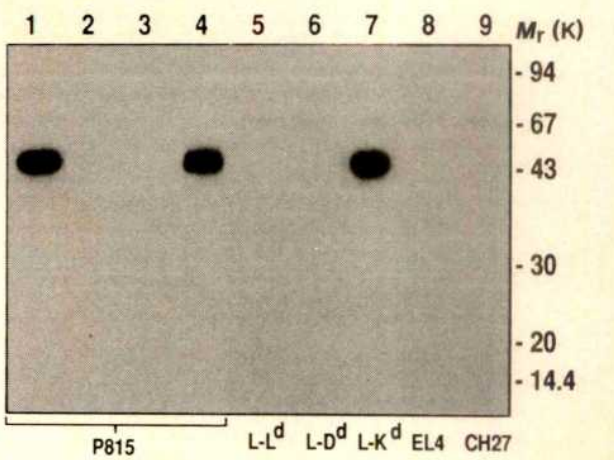

FIG. 2 The IASA–YIPSAEK(biotin)I conjugate selectively labels the K^d molecule. P815 cells (H-2^d, lanes 1–4), L cells (H-2^k) transfected with *L*^{d} (lane 5), *D*^{d} (lane 6) or *K*^{d} (lane 7) genes, EL-4 cells (H-2^b, lane 8) and CH-27 cells (H-2^k, lane 9) were incubated with [^{125}I]IASA–YIPSAEK(biotin)I and then irradiated with ultraviolet light. Cell lysates were analysed directly by SDS–PAGE (lanes 1, 5–9). In addition, lysates from labelled P815 cells were immunoprecipited with monoclonal antibody specific for L^d (lane 2), D^d (lane 3) and K^d (lane 4) and then analysed by SDS–PAGE. Migration of size markers is shown on the right.
METHODS. Cells were washed twice in phosphate-buffered saline (PBS) and resuspended in DMEM supplemented with HEPES (20 mM), gentamycin (40 μg ml^{-1}) and FCS (0.5%) at 2 × 10^6 cell per ml. Cells (2 × 10^6) were incubated with [^{125}I]IASA–YIPSAEK(biotin)I (1 × 10^7 c.p.m. in 10 μl PBS) in 6-well plates (Costar) at 37 °C for 6 h and then irradiated with ultraviolet light at 4 °C for 10 min (15 watt lamp with an emission maximum at 312 nm; Bioblock Scientific, France). [^{125}I]IASA–YIPSAEK(biotin)I was prepared using [^{125}I]IASA–ONSu which was prepared as described[29,30], had a specific activity of 1,700–2,300 Ci per mmol and was used at a concentration of ~3 × 10^{-9} M. Labelled cells were washed twice in cold DMEM containing 5% FCS and once in PBS, then boiled for 5 min in 40 μl sample-reducing buffer. Alternatively, cells on ice were solubilized with NP-40 (0.7%) in the presence of iodacetamide (10 mM), PMSF (1 mM) and leupeptin (10 μg ml^{-1}), and immunoprecipitated[29,30]. The anti-K^d monoclonal antibody was SF 1.1.1.1, the anti-D^d was 34–4–20S and the anti-L^d was 28-14-8S. The hybridomas producing these monoclonal antibodies were from American type culture collection (Rockville, ML). SDS–PAGE was on linear 10% polyacrylamide gels under reducing conditions. Cell-surface expression of L^d, D^d and K^d on the L-cell transfectants[31,32] was verified by flow cytometric analysis to be at least as high as that on P815 (H-2^d) cells (not shown). For P815 cells, about 300–500 K^d molecules per cell became photolabelled under these conditions.

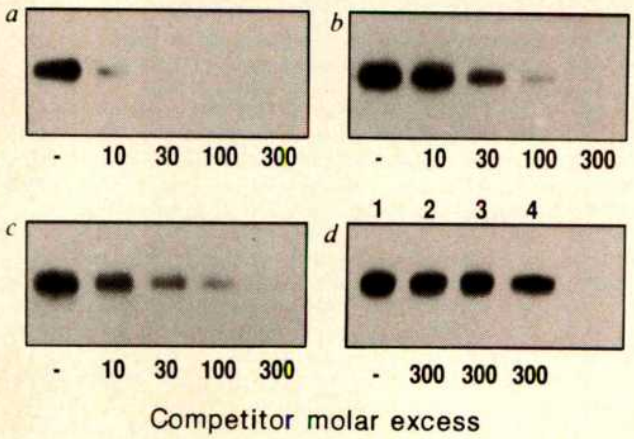

FIG. 3 Competition of the photoaffinity labelling of K^d on P815 cells by different antigenic peptides. P815 cells were incubated as described for Fig. 2 with [^{125}I] IASA-YIPSAEK(biotin)I in the absence or presence of the indicated molar fold excess of peptides CS P.b. 253–260 (*a*), P198^{-}.14–24 (*b*), AYP5TLA (*c*), LCMV NP 118–132 (*d*, 2), Ad5 E1a 234–243 (*d*, 3) and P91A^{-}.12–24 (*d*, 4). After irradiation with ultraviolet light, the material immunoprecipitated by the anti-K^d SF1.1.1 was analysed by SDS–PAGE as described for Fig. 2.

intracellularly, or to newly synthesized 'empty' class I molecules as they emerge at the cell surface.

Photoactivation of the IASA group involves the formation of a short-lived and highly reactive intermediate, phenylnitrene, which can then form covalent bonds with residues on the target protein[28]. The inclusion of a biotin moiety in the photoprobe, as in the IASA–YIPSAEK(biotin)I compound, should facilitate investigations of the subcellular localization and mechanism of peptide–MHC interactions. Furthermore, studies with class II molecules[29,30] have demonstrated that photoaffinity labelling can be useful for identifying antigen contact sites on MHC molecules, and it may now be possible to apply the same approach to MHC class I molecules. □

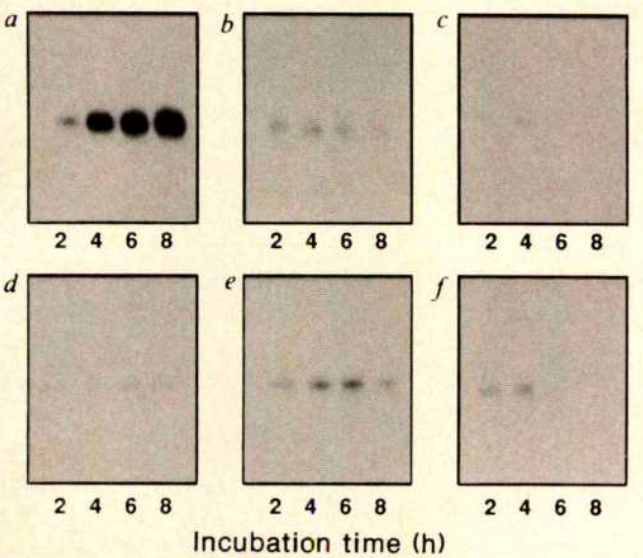

FIG. 4 Photoaffinity labelling of K^d on P815 cells in the presence or absence of different inhibitors. Cells were incubated with [^{125}I]IASA-YIPSAEK(biotin)I for the indicated periods of time at 4 °C (*b*) or at 37 °C (*a*, *c*–*f*) in the absence (*a*) or presence of sodium azide (0.02%) and 2-deoxyglucose (10 mM) (*c*), cycloheximide (10 μg ml^{-1}) (*d*), or brefeldin A (1 μg ml^{-1}) (*e*). Alternatively, the cells were first solubilized with MEGA 8 (30 mM) and MEGA 9 (30 mM) detergents plus a mixture of enzyme inhibitors (see methods for Fig. 2) immediately before incubation with the photoprobe (*f*). Following ultraviolet irradiation, the material immunoprecipitated with the anti-K^d antibody SF1.1.1.1 was analysed by SDS-PAGE as described for Fig. 2.

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Peptide binding to empty HLA-B27 molecules of viable human cells

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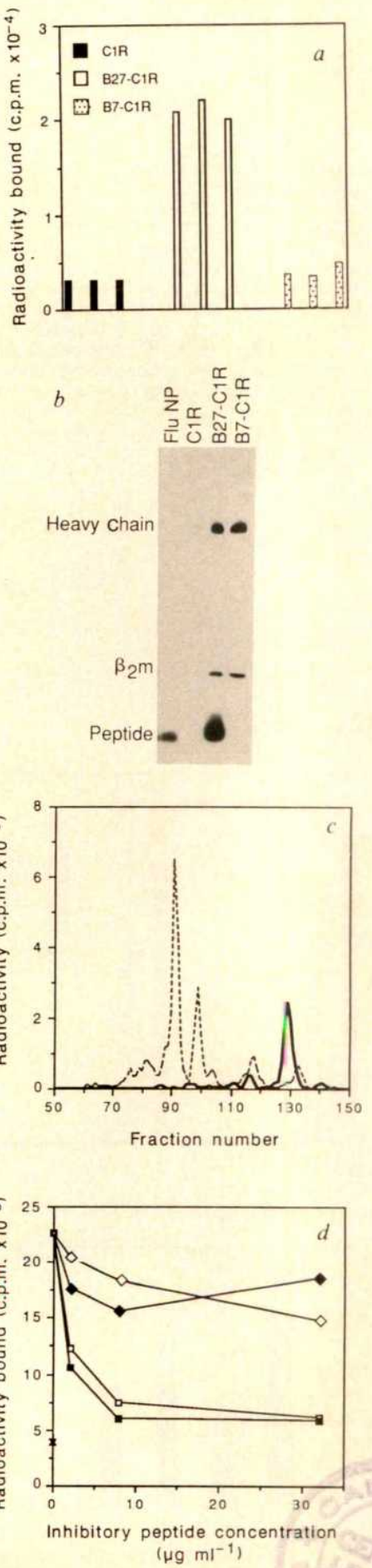
INTRACELLULAR binding of antigenic peptides by polymorphic class I major histocompatibility complex molecules creates the ligands recognized by receptors of CD8$^+$ T cells[1]. Previously described *in vitro* assays of peptide binding to class I molecules have been limited by either the low proportion of accessible binding sites or the lack of allelic specificity[2–6]. Here we describe a system in which the human class I molecule HLA-B27 binds considerable amounts of an influenza peptide with precise allelic discrimination. Binding requires viable cells, is stimulated by γ-interferon and is inhibited by brefeldin A. Our results are consistent with the presence of fairly stable 'empty' HLA-B27 molecules at the cell surface. By contrast, analysis of the binding of a second influenza peptide indicates that empty HLA-Aw68 molecules are relatively short-lived. We speculate that HLA-B27 might bind extracellular peptides *in vivo* and that this property could underlie its association with autoimmune disease.

HLA-B27 presents a synthetic peptide (NP1) derived from residues 383–394 of the influenza nucleoprotein to T cells[7]. We cultured radioiodinated NP1 with HLA-B27-transfected C1R cells (an HLA-A, B-negative human B-cell line[8]) which were concomitantly radiolabelled with [^{35}S]methionine. Measurable ^{125}I-labelled peptide could be immunoprecipitated with class I molecules (Fig. 1*a*); all three components (HLA-B27 heavy

FIG. 1 *a*, *b*, NP1 peptide reproducibly coprecipitates with HLA-B*2705. I^{125}-NP1 was cultured with either untransfected C1R cells(solid), B27-C1R transfectants(open), or B7-C1R(stipple) cells, and the HLA-class I molecules immunoprecipitated and counted for radioactivity. *b*, 12.5% SDS-PAGE of individual precipitates. *c*, Reverse-phase HPLC analysis of I^{125}-NP1(dotted line) and peptide eluted from HLA-B*2705 immunoprecipitates (solid line). *d*, Peptide binding can be inhibited by an excess of HLA-B*2705 restricted peptides. I^{125}-NP1 ($2\mu g\ ml^{-1}$) was incubated with B27-C1R or C1R cells (cross) plus cold peptides. These inhibitors and their amino-acid sequences in the one-letter amino-acid code were NP1, SRYWAIRTRSGG (open squares); HIV*gag* (265–279) peptide, KRWIILGLNKIVRMYC (solid squares); FluNP (89–101), PKKTGGPIYKRVD (open diamonds); or FluNP (335–349), SAAFEDLRVLSFIRGY (solid diamonds). The carboxy-terminal residues of HIV-*gag* peptide and FluNP (335–349) were not a part of the viral sequences. METHODS. Iodination was by the chloramine T method, the product being separated on a Sep-Pak C18 cartridge. Typically, 2–8% of molecules were labelled. HLA class-I expression was determined on the day of each assay by immunofluorescence. Cells were preincubated overnight with 500 units per ml γ-interferon. I^{125}-NP1 peptide was added to 2.5×10^6 cells in 0.5 ml methionine-free RPMI plus 1% BSA, 500 units per ml γ-interferon and $60\ \mu Ci\ ml^{-1}$ ^{35}S-methionine, and incubated for 6 h at 37 °C. Cells were washed and lysed, and the class I molecules immunoprecipitated and washed as described[19]. Lysates were precleared twice for 30 min with 125 μl washed Staph A cells plus 5μl normal rabbit serum. Immunoprecipitation was effected with 10 μg purified W6/32 (ref. 29) plus 50 μl Staph A cells and incubation for 30 min on ice. Precipitated radioactivity was measured directly with a LKB 1260 Multigamma II Gamma Counter. Peptide was eluted from immunoprecipitates in 70% acetonitrile, 0.3% trifluoroacetic acid (TFA), then dried, redissolved in 0.1% TFA and loaded onto an Altex Ultrasphere ODS C18 column, from which it was eluted with a 0–80% acetonitrile gradient in 0.1% TFA.

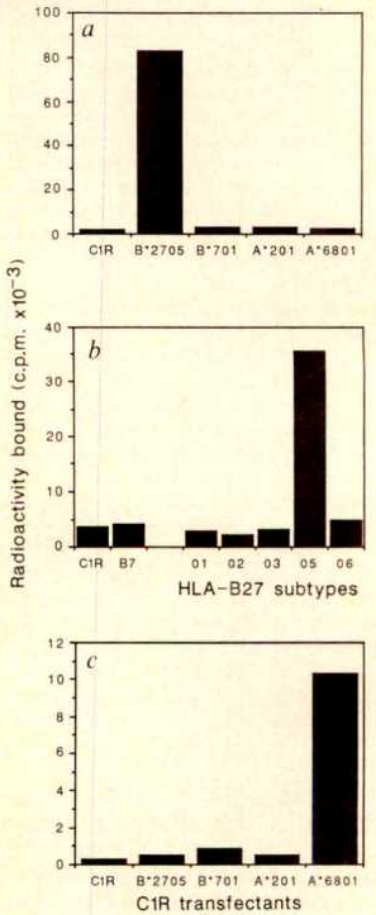


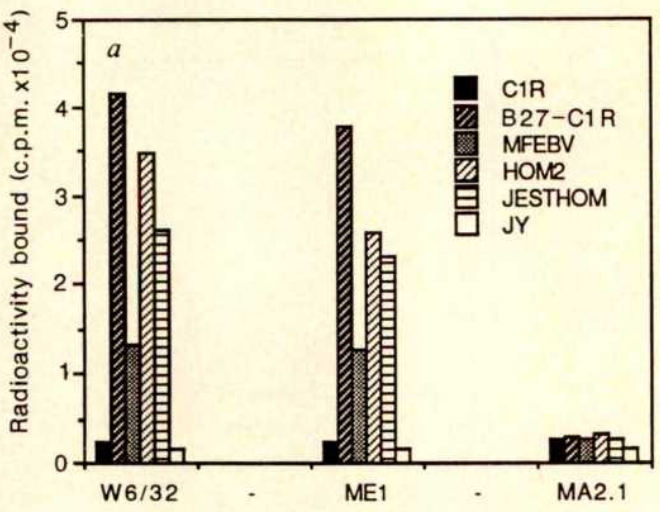
chain, β2-microglobulin (β2m) and NP1) were resolved by SDS-polyacrylamide gel electrophoresis (Fig. 1*b*). Reverse-phase HPLC of the bound radioiodinated peptide revealed a single dominant peak that was distinct from the main components of the incubated ^{125}I-labelled peptide (Fig. 1*c*), indicating that either HLA-B27 is selecting a minor component of the added peptide or that proteolytic processing of the peptide is occurring[9–11].

This binding had peptide and HLA specificity corresponding to the patterns of cytotoxic T lymphocyte recognition[7]. It was inhibited by non-radioactive preparations of NP1 and an HLA-B27-presented human immunodeficiency virus *gag* peptide[12], but not by other peptides (Fig. 1*d*). Similarly, ^{125}I-NP1 only bound to the common subtype of HLA-B27 (B*2705) and not to seven other HLA-A, B molecules (Fig. 2*a*, *b*). Binding was not due to nonspecific 'stickiness' of HLA-B27 because a second peptide (NP2) derived from residues 335–349 of the influenza nucleoprotein[13] bound to HLA-Aw68 (A*6801) but not to HLA-B27 (Fig. 2*c*). Neither was binding peculiar to transfectants of the mutant C1R cell line as non-mutant B-cell lines gave similar results (Fig. 3*a*, *b*). That binding is not simply a function of the cell-surface abundance of HLA-B27 molecules was shown by the increased binding to C1R transfectants in comparison to normal B-cell lines expressing equivalent or greater amounts of cell-surface HLA-B27, and also by the 50-100% increase in peptide binding produced by γ-interferon compared with its minimal effect (±10%) on cell-surface expression (Fig. 3*c*). No change in peptide binding was caused by the presence of serum or by the addition of purified human β2m (data not shown), thus β2m exchange does not seem to be a significant factor in the peptide-binding mechanism[14–16].

Inhibition by cycloheximide and brefeldin A (refs 17, 18) showed that biosynthesis and exocytic transport, respectively, of class I polypeptides are important in this assay. Preincubation and inclusion of either drug in the assay reduced binding of NP1 to HLA-B27 by ~50%, and of NP2 to HLA-Aw68 by 80–90% (Fig. 4). Binding of NP1 to HLA-B27 is not due to reactions taking place after cell culture in the detergent cell lysate, as it was not blocked by addition of excess 'cold' peptide after cell culture and before lysis (Fig. 4*b*,ii). Neither was binding reconstituted by addition of ^{125}I-NP1, nor of C1R cells loaded

FIG. 2 Allelic specificity of peptide binding. I^{125}-NP1 peptide binding to C1R cells expressing *a*, different HLA-A,B alleles and *b*, different HLA-B27 subtypes[30]; *c* I^{125}-NP2 binding to C1R cells expressing different class I alleles. METHODS. B*701-C1R, A*201-C1R and A*6801-C1R were stable transfectants. HLA-B27 sybtypes were produced by site-directed mutagenesis of the HLA-B*2705 genomic sequence by the method of Kunkel[31] using the Biorad (Richmond, CA) Muta-gene *in vitro* mutagenesis kit. The following mutations were made: HLA-B*2701 (74D→Y; 77D→N; 81L→A); HLA-B*2702 (77D→N; 80T→A; 81L→A); HLA-B*2703 (59Y→H); HLA-B*2706 (77D→S; 114H→D; 116D→Y; 152V→E) and were based on the sequences described in ref. 30. Mutant and wild-type genes were subcloned into the vector pHEBO and electroporated into C1R cells as described[32]. HLA class-I expression was determined by immunofluorescence: all transfectants gave comparable levels on the day of assay. Isoelectric focusing gel electrophoresis confirmed the mutant gene products had isoelectric points identical to the naturally occurring HLA-B27 subtypes.





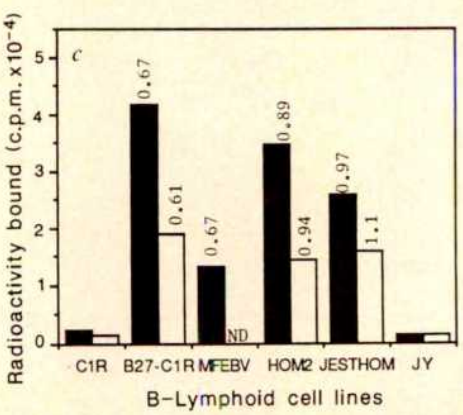


FIG. 3 *a*, Peptide binding to Epstein-Barr virus (EBV)-transformed human B cell lines. The binding of I^{125}-NP1 peptide to HLA-B*2705 positive (MF, HOM2 and JESTHOM) and HLA-B*2705 negative (JY) B-cell lines was assessed. B27-C1R and C1R cells were included as positive and negative controls. *b*, Reducing 12.5% SDS-PAGE of immunoprecipitates from EBV-transformed B-cell lines. *c*, Effect of γ-interferon on I^{125}-NP1 binding to B-cell lines. Cells were incubated either in the presence of 500 units per ml γ-interferon (solid bars) or in its absence (open bars) from both the overnight preincubation and the peptide incubation. Numbers represent the HLA-B*2705 cell-surface expression as determined by quantitative radioimmunoassay ($\times 10^6$) (ref. 33).

METHODS. HLA types were: C1R (–, –, Cw4); B27-C1R (–, B*2705, Cw4); MF (A1, A2, B*2705, Bw54, Cw1); HOM2 (A3. B*2705, Cw1); JESTHOM (A2. B*2705, Cw1) and JY (A2, B7). HLA class I was immunoprecipitated with either W6/32 (monomorphic), ME1 (specific for HLA-B27, B7, Bw42 and Bw22) (ref. 34) or MA2.1 (specific for HLA-A2 and B17) (ref. 35).

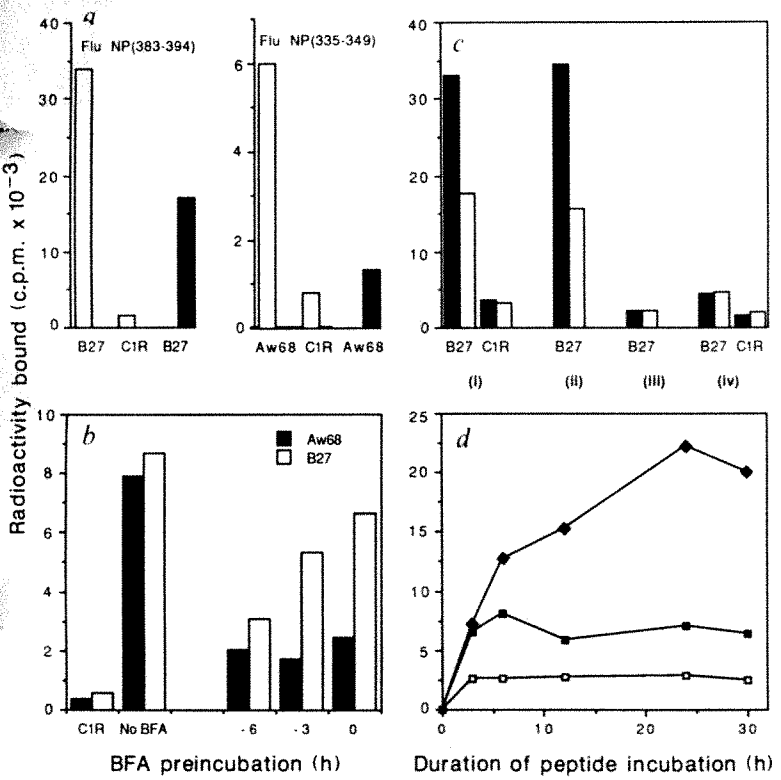


FIG. 4 *a*, Effect of cycloheximide (solid bars) on I^{125}-NP1 binding to HLA-B*2705 and I^{125}-NP2 binding to HLA-A*6801. *b*, Effect of brefeldin A (BFA), given for varying preincubation periods, on I^{125}-NP1 binding to HLA-B*2705 (open columns) and I^{125}-NP2 binding to HLA-A*6801 (solid columns). *c*, I^{125}-NP1 binding, in the presence (open bars) or absence (solid bars) of brefeldin A: (i) control incubation with 4 μg ml^{-1} I^{125}-NP1; (ii) 10 μg non-radioactive NP1 added to washed cells just before lysis; (iii) B27-C1R cells mock-treated without peptide for 6 h at 37 °C, washed, and then mixed with washed I^{125}-NP1 laden C1R cells just before lysis; (iv) B27-C1R or C1R cells mock-labelled without peptide, washed and 100 ng I^{125}-NP1 added just before cell lysis. *d*, Binding of I^{125}-NP1 as a function of time. Peptide binding at 20 μg ml^{-1} to untreated B27-C1R transfectants (solid diamonds), brefeldin A-treated B27-C1R transfectants (solid squares) and to C1R cells (open squares).

METHODS. Cycloheximide was added to 20 μM one hour before I^{125}-NP1. SDS-PAGE confirmed the complete inhibition of ^{35}S-methionine incorporation into class I molecules. Brefeldin A was resuspended at 10 mg ml^{-1} in methanol and stored at −70 °C. A titration determined that maximum inhibition of peptide binding, and also of sialation of HLA class I (as determined by isoelectric focusing gel electrophoresis) occurred at concentrations of 1 μg ml^{-1}. Cells were pretreated for 2 h with 10 μg ml^{-1} brefeldin A before addition of I^{125}-NP1 and the standard 6-h culture in the experiments shown in Fig. 4*c* and *d*. In *c*, iv, 100ng I^{125}-NP1 was used as this represented the maximum percentage (5%) of peptide generally carried over in cell lysates after washing. The percentage surface class I labelled was determined from: Average number of peptide molecules per cell = (total no. peptides bound)/(2.5 × 10^6 cells); and total no. of peptides bound = (specific c.p.m. bound) × (moles peptide × Avagadro's no.)/(total c.p.m. I^{125}-NP1).

with ^{125}I-NP1, to mock-cultured B27-C1R cells before lysis (Fig. 4*c*, iii and iv).

In the presence of brefeldin A, maximal binding of ^{125}I-NP1 to HLA-B27 was reached within 6 hours. Without brefeldin A, a similar initial component was observed, followed by a slower component that attained a higher maximum after 24 hours. This level corresponds to 2.4×10^5 peptide-loaded HLA-B27 molecules per cell, about 36% of the total cell-surface HLA-B27 molecules detected by radioimmune assay. These results indicate that exocytosis of newly synthesized HLA-B27 is the rate-limiting step in peptide binding.

Our results are consistent with the interpretation that the observed binding is to functionally empty class I HLA molecules[19–24]. We have not demonstrated that binding is at the cell surface, and an alternative intracellular site for peptide loading is not ruled out. The binding detected in the presence of brefeldin A may reflect a relative stability of empty HLA-B27 molecules. Increasing the period of preincubation with brefeldin A diminishes the magnitude of this binding, but it was still detectable even after a 6-hour preincubation. Peptide binding to HLA-Aw68 was more sensitive to brefeldin A and cycloheximide, indicating empty HLA-B27 molecules are longer lived than empty HLA-Aw68 molecules. Comparison with previous reports suggests that empty HLA-B27 molecules are also more stable than empty H-2 molecules[19–24]. A further distinction is that culture at reduced temperature (26 °C) does not increase peptide binding to empty HLA-B27 molecules. By contrast, overnight preincubation with γ-interferon increases peptide binding even when brefeldin A is added later (data not shown). As previously proposed[25], this suggests that γ-interferon facilitates assembly and exocytosis of class I molecules—in this case, empty class I molecules.

With this 'peptide feeding' assay we find that a significant proportion of cell-associated HLA-B27 molecules bind peptide with allelic specificity. Peptides seem to bind to empty HLA-B27 molecules that are relatively stable under physiological conditions. The possibility that certain class I molecules present extracellular peptides to T cells *in vivo* should therefore be considered. For HLA-B27, this property might underlie the strong association of this allele with ankylosing spondylitis and other seronegative spondylarthropathies (refs 26 and 27; reviewed in ref. 28). □

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Acquisition of the human adeno-associated virus type-2 *rep* gene by human herpesvirus type-6

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HUMAN herpesvirus type-6 (HHV-6) is a recently isolated herpesvirus which is highly prevalent in adult populations around the world[1–3]. HHV-6 was first isolated from the peripheral blood of six individuals with lymphoproliferative disorders, two of whom were also infected with human immunodeficiency virus[1]. HHV-6, in common with other herpesviruses, transactivates the HIV long terminal repeat linked to reporter genes[4,5] and has in addition been shown to accelerate HIV gene expression and CD4 cell death in cultures co-infected with both viruses[6]. The virus is tropic for CD4$^+$ lymphocytes[7,8] and persists in the peripheral blood of most seropositive individuals[9]. We have now identified a gene in HHV-6 encoding a 490-amino-acid polypeptide homologous to the human adeno-associated virus type-2 (AAV-2) *rep* gene[10]. This gene has an essential role in AAV-2 DNA replication[11,12], can *trans*-regulate homologous and heterologous gene expression[13–17], and inhibits cellular transformation[18]. The acquisition of *rep* by HHV-6 could be due to natural transfer of genetic information between DNA viruses of eukaryotes and is likely to have important consequences for the life-cycle of HHV-6 and for the host CD4 cell.

The genomic structure of a Ugandan isolate of HHV-6 (strain U1102) has been determined and consists of a 141-kilobase (kb), largely unique sequence flanked by direct repeat units of roughly 10 kb[19]. Sequencing shows that HHV-6 is closely phylogenetically related to human cytomegalovirus[20] and that the genetic organization of these two herpesviruses is highly conserved (B.J.T. *et al.*, manuscript submitted). Nucleotide analysis of a 12-kb *Hin*dIII fragment of HHV-6 U1102, which lies proximal to the right-hand terminal repeat of the viral genome[19] (Fig. 1*a*), has identified a 490-amino-acid open reading frame (Fig. 1*b*) homologous to the *rep* 68/78 gene product of the human helper-dependent parvovirus AAV-2 (ref. 10). The product of the HHV-6 *rep* gene homologue (HHV-6 PH) was more closely related to the AAV-2 gene product than were the equivalent non-structural NS1/*rep* proteins encoded by the autonomous parvoviruses (Table 1). An alignment of the predicted translation product of HHV-6 PH with the AAV-2 Rep 68/78 polypeptide is shown in Fig. 2. The proteins share a 24% identity across the entire length of the 490-amino-acid HHV-6 PH reading frame. The close relationship between the two proteins is most clearly illustrated by a DIAGON[21] plot, compared with a plot showing the relationship of the AAV-2 Rep protein to the equivalent protein encoded by human autonomous parvovirus B19 (Fig. 3*a* and *b*). The HHV-6 PH product is more closely related to the AAV-2 protein throughout its length than are the helper-dependent and autonomous parvovirus proteins to each other. All parvovirus Rep/NS1 proteins contain a well conserved 145-amino-acid domain which has significant homology to the T antigens of polyomavirus and simian virus 40 and the E1 proteins of papillomaviruses[22]. This domain contains a consensus purine nucleotide triphosphate binding site essential to the role of these proteins in DNA replication[23]. This consensus is conserved in HHV-6 PH. A multiple alignment of this region of the HHV-6 PH product with AAV-2 and autonomous parvovirus Rep/NS1 proteins is shown in Fig. 3*c*.

We have no direct evidence for the expression of HHV-6 PH. There is, however, no evidence for the presence of pseudogenes in the unique regions of other characterized herpesviruses and the codon usage in this *orf* conforms to that of other recognized HHV-6 genes (ref. 20, and S.E. and B.J.T., unpublished observations). The first ATG codon conforms to the Kozak consensus[24]; there is a TATA box 28 base pairs (bp) upstream of this methionine codon and a polyadenylation signal sequence AATAAA at position 1,817 (Fig. 1). The HHV-6 PH is therefore highly likely to be expressed.

TABLE 1 Homology between HHV-6 PH, AAV-2 and equivalent proteins

	HHV-6	AAV-2	B19	BPV	MVM
HHV-6	*	619	315	235	210
AAV-2		*	598	417	390
B19			*	399	289
BPV				*	319
MVM					*

FASTA scores obtained by searching the PIR database with the amino-acid sequences of the HHV-6 PH ORF, AAV-2 *rep* 68/78 gene and the equivalent non-structural NS1 proteins of human (B19), bovine (BPV) and murine (MVM) autonomous parvoviruses (refs 28, 30 and 31, respectively). Searches were made using the FASTA program[39] using a Ktuple of 1.

Herpesvirus genomes are known to contain genes related to ancestral cellular counterparts[25–27]. These genes lack the introns of their cellular homologues, suggesting acquisition by the reverse transcription of mature messenger RNA. The region of the AAV-2 *rep* gene product to which HHV-6 PH is homologous is encoded by unspliced mRNA. The helper-dependent and autonomous parvovirus Rep proteins appear to constitute a unique family of regulatory proteins and no cellular homologue has been identified. The nucleotide sequences of several herpesvirus (summarized in ref. 19) and parvovirus[28–31] genomes do not indicate a common ancestral origin for these two distinct lineages and in particular no evidence of a *rep* gene homologue in other herpesviruses. The HHV-6 PH protein is more closely related to the AAV-2 *rep* 68/78 gene product than are the Rep proteins of the other sequenced parvoviruses, and DIAGON[21] comparisons of the two proteins indicate that this similarity is not limited to recognized functional domains (Fig. 3*a*, *b*). Although the homology may be due to a common ancestral origin or convergence in sequence evolution, the nature of the sequence similarity between the HHV-6 PH protein and the product of the AAV-2 *rep* 68/78 gene indicates that HHV-6 has acquired this gene by direct transfer from AAV-2. The properties of AAV-2 suggest a way in which this could take place. Adeno-associated viruses do not normally replicate in cells unless they have been coinfected with a member of either the adeno- or herpesvirus families[32]. In the absence of helper virus, adeno-associated viruses establish latent infection of the host cell as an integrated provirus[33]. HHV-6, which is likely to provide such helper function, could therefore have 'captured' the *rep* gene by a mechanism of nonhomologous recombination with AAV-2 replicative intermediates, or integrated provirus.

The AAV-2 *rep* gene encodes at least four overlapping polypeptides produced by the use of two promoters and alternative splicing and identified by their apparent molecular size on acrylamide gels as Rep 78, Rep 68, Rep 52 and Rep 40[10,11,34]. Rep 78 is encoded by a 4.2-kb unspliced transcript, and the closely related Rep 68 from a singly spliced 3.9-kb transcript. Rep 68 binds to the AAV-2 origin of replication in a sequence-dependent manner and has a well defined ATP-dependent site-specific endonuclease and DNA helicase activity[11]. The region of homology with HHV-6 includes almost all of the Rep 68 protein. Mutations in the region of the *rep* 68/78 gene homologous to HHV-6 PH show that the gene has other important functions[12–16]. AAV-2 *rep* gene products act as positive and negative regulators of both homologous[13,14,16] and heterologous[15] promoters, including the HIV long terminal

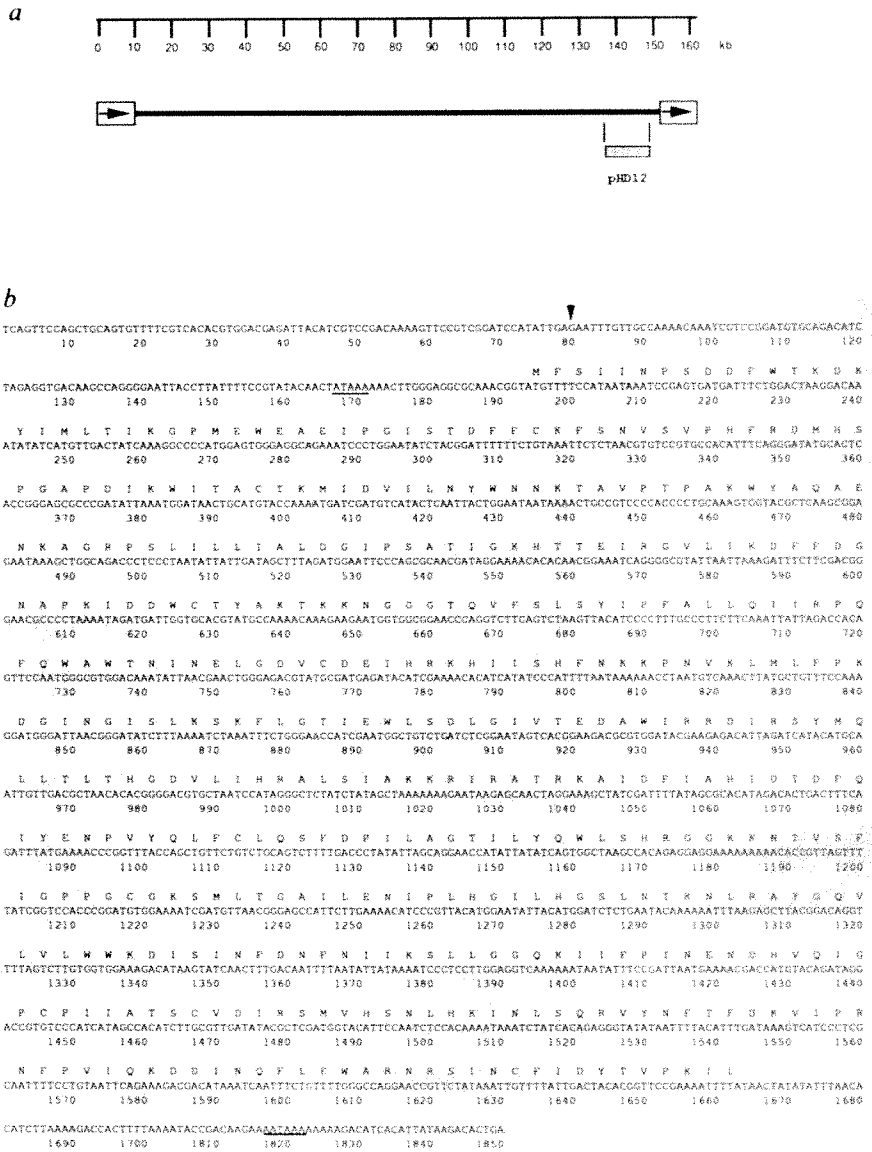
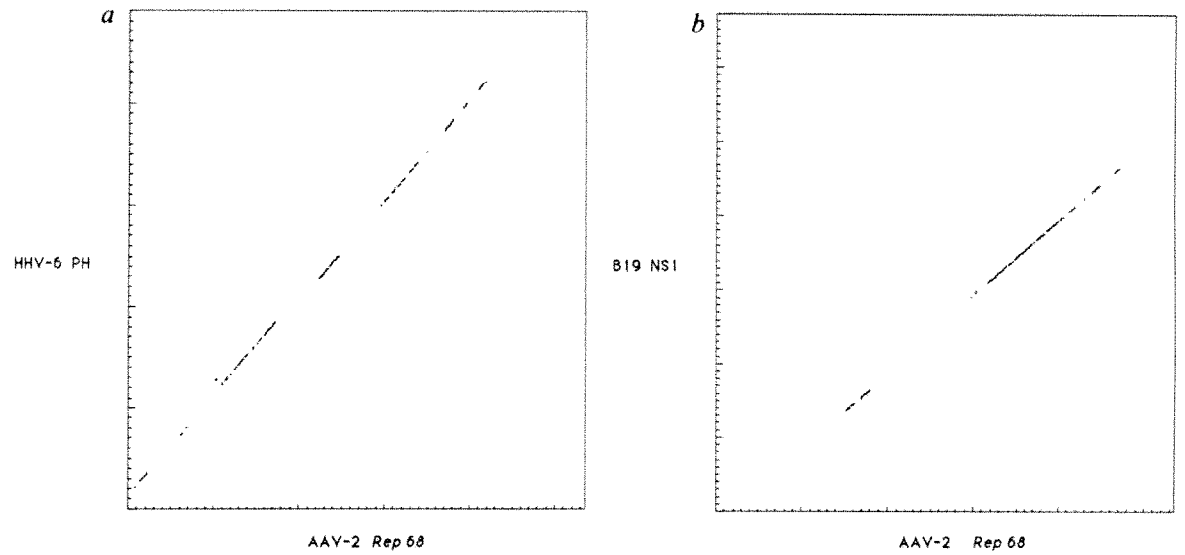


FIG. 1 *a*, Structure of the HHV-6 strain U1102 genome. The single black line represents 141 kbp of unique sequence DNA flanked by large direct repeats of 10 kbp[19]. The location of the 12-kbp *Hin*dIII restriction fragment sequenced in this study is indicated. *b*, Nucleotide and predicted protein sequence (single-letter amino-acid code) of the HHV-6 PH open reading frame. An arrow marks the start of the open reading frame. A TATA box which lies 28 bp upstream from the first Kozak methionine and an AATAAA polyadenylation signal sequence which starts at nucleotide number 1,817 are underlined. The sequence was determined from the HHV-6 strain U1102 12-kbp *Hin*dIII-C fragment cloned into pUC13. Sequences of both strands were determined by the dideoxy chain termination method[37] and analysed using the NIP and SIP programs[38].

FIG. 2 Amino-acid sequence homology (single-letter amino-acid code) shared between the HHV-6 PH open reading frame and the AAV-2 *rep* 68/78 gene product[10]. Boxed residues indicate the identity between the two sequences. The entire 490-amino-acid HHV-6 gene sequence is shown aligned with the first 490 amino acids common to both the Rep 78 and Rep 68 AAV-2 polypeptides. The sequences were aligned manually with the aid of the FASTA program library search alignment output[39].



c

```
                                   I                                  II
CPV       392 KRNTVLFHGPASTGKSIIAQAIAQAVGNVGCVNAANVNFPFNDCTNKNLIWIEEAGNFGQQVNQFKAICSGQTIRIDQKGKGSKQIEPTPVIMTT
MVM       440 KRNTVLFHGPASTGKSIIAQAIAQAVGNVGCVNAANVNFPFNDCTNKNLIWVEEAGNFGQQVNQFKAICSGQTIRIDQKGKGSKQIEPTPVIMTT
BPV       302 KRNSTLFYGPASTGKTNLAKAICHAVGLVGCVNHNNKQFPFNDAPNKHIIWWEECIMTTDVVEAAKCVLGGTHVRVDVKHKDSRELPQIPVLLSS
B19       320 KKNTLWFVGPPSTGKTNLAMAIAKSVPVYGMVNWNNENFPFNDVADKSLVVWDEGIIKSTIVEAAKAILGGQPTRVDQKMRGSVAVPGVPVVITS
AAV-2     325 KRNTIWLFGPATTGKTNIAEAIAHTVPFYGCVNWTNENFPFNDCVDKMVIWWEEGKMTAKVVESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTS
HHV-6 PH 330 KKNTVSFIGPPGCGKSMLIGALELNIPLHGILHGSLNTKNLRAVGQVLVEWWKDISINEDNFNIIKSLLGGQKIIFPINENDHVQIGPCPIIATS
             * *- - ** -** - ** - * - - ----- - - - - - * * - - - - * - -
```

FIG. 3 Comparison of the protein sequences of HHV-6 PH open reading frame and AAV-2 *rep* 68/78 gene[10] (*a*) and human autonomous parvovirus B19 NS1 protein[28] and AAV-2 *rep* 68/78 gene (*b*) using the DIAGON program[21]. *c*, Multiple alignment of a 95-amino-acid domain in a 145-amino-acid block that is well-conserved in all mammalian autonomous and helper dependent parvoviruses. The sequences used are those of the canine parvovirus (CPV)[29], bovine parvovirus (BPV)[30], minute virus of mice (MVM)[31], human parvovirus B19 (ref. 28), the human helper-dependent parvovirus AAV-2 and the HHV-6 PH open reading frame. The two boxed domains represent motifs common to nucleases, which are separated here by a spacer region of 23 amino acids[40]. Region I contains a consensus purine nucleotide-binding site ((G/A) XXXXGK(S/T))[23]. Identical amino acids conserved among all the protein sequences are indicated by an asterisk, and where all but one of the sequences share identical amino acids is indicated by a dash.

repeat[17]. The *rep* 68/78 specifically inhibits DNA amplification[35] induced by herpes simplex virus, and cellular transformation by papillomaviruses[18]. The C termini of the Rep 68/78 proteins, which are not represented in the HHV-6 homologue, are not required for inhibition of DNA amplification (R. Heilbronn, personal communication). Furthermore, the parvovirus B19 Rep protein, which is less closely related to the AAV-2 *rep* gene product than is HHV-6 PH, can correctly replicate hybrid genomes in which AAV-2 origins of replication are substituted for B19 origins[36]. The conservation between the HHV-6 PH and the AAV-2 *rep* gene product therefore suggests that HHV-6 PH has a similar range of functions which are advantageous to the survival of HHV-6 in the host. The expression by this ubiquitous herpesvirus of a gene that modulates heterologous gene expression and cellular transformation may have important consequences for the host cell. □

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Microbiological characterizations by FT-IR spectroscopy

Dieter Naumann, Dieter Helm and Harald Labischinski

Infrared signals of microorganisms are highly specific fingerprint-like patterns that can be used for probing the identity of microorganisms. The simplicity and versatility of Fourier-transform infrared spectroscopy (FT-IR) makes it a versatile technique for rapid differentiation, classification, identification and large-scale screening at the subspecies level.

THE detection and characterization of microorganisms by physical techniques promises to be of great value because of the inherent sensitivity, rapidity and potential for complete computerization. Spectroscopic techniques, in particular, are extremely specific and provide a wealth of qualitative and quantitative information about a given sample. The infrared spectrum of any compound is known to express a unique 'fingerprint' and it is this characteristic that enables IR-spectroscopy to be used in the identification of unknown samples using spectral data libraries (for example, in forensic medicine and the pharmaceutical industry). The advent of modern interferometric and Fourier-transform techniques has augmented the specifications of IR- spectroscopy and, thus, paved the way for sophisticated and novel applications of IR in the field of biomedical research.

A joint project was undertaken between 1986 and 1989 to develop efficient methods for the FT-IR analysis of intact bacteria. The project, conducted under the auspices of the Ministry of Research and Technology and the Ministry of Health of Germany, was carried out by researchers at the Robert Koch-Institut of the Federal Health Office in Berlin in collaboration with a manufacturer of FT-IR spectrometers (Bruker, Analytische Meßtechnik, Karlsruhe, Germany). Drawing upon the practical knowledge obtained to date, the prototype of a dedicated instrument, which will be a 'workhorse' for microbiological characterization by FT-IR, is now under development at Bruker and will be available for testing by several laboratories in a joint pilot study in early 1992.

Information basis

The FT-IR technique establishes spectral fingerprints of intact bacteria. These patterns comprise the vibrational characters of all cell constituents, that is, DNA/RNA, protein, membrane and cell-wall components. Consequently, FT-IR probes the total composition of a given organism in a single experiment. As all cell compounds depend on the expression of smaller or larger parts of the genome, the spectra display in a specific way a phenetic and a genetic fingerprint of the microorganisms under study. This is why the selectivity of the new technique is extremely high, allowing differentiations down to the subspecies and, in most cases, even to the strain and/or serogroup/serotype level[1–4].

Owing to the multitude of cellular components, broad and superimposed spectral bands are observed within the mid-infrared range (~ 4,000–600 reciprocal wavelengths = wavenumbers [cm^{-1}]). Some spectral subranges are dominated by particular cell components, for example, fatty acids, polysaccharides or proteins. In order to extract specific information from the broad spectral contour, it is advantageous to analyse microbial FT-IR spectra by considering a defined combination of spectral windows and to apply sophisticated mathematical filter techniques (see Fig. 1). As an example of the discriminative power of the method, figure 2 presents the FT-IR classification of some *Pseudomonas* spp. determined by a cluster analysis approach. The dendrogram of figure 2 reveals a hitherto unknown subdivision of the *Pseudomonas fluorescens* sp. in two main groups, the members of which can be further differentiated down to the strain level within a few minutes of measurement and evaluation.

Experimental procedures

As intact cells are tested, complicated and time-consuming procedures for decomposing cells into single chemical or structural components are avoided. Consequently, simple and uniform procedures are available that are applicable to all bacteria that can be grown in culture; standardization is no longer a crucial step. In short, small amounts of cells (~ 10–60 μg) grown under standardized conditions are transferred from the culture plate to an IR-sample holder using a platinum loop. After a short drying procedure, the sample holder is exposed to the IR-beam, the spectrum is recorded and automatically stored in a data file. The characterization of microorganisms is accomplished

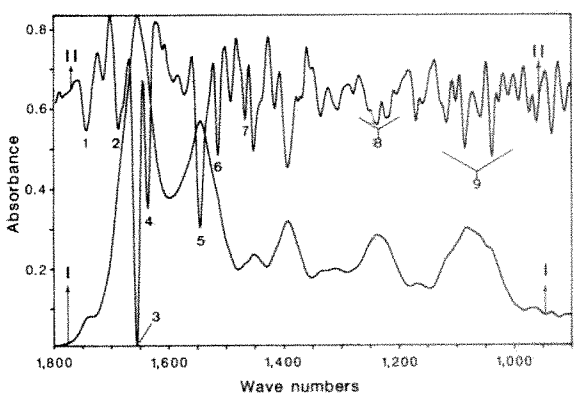

FIG. 1 Whole-cell Fourier-transform infrared (FT-IR) spectra of *Staphylococcus aureus* strain Pelzer. I, Original spectrum, and II, the filtered (resolution enhanced) spectrum as calculated from I. The numbered peaks denote IR bands that can be assigned to specific structural cell components[1,3,4].

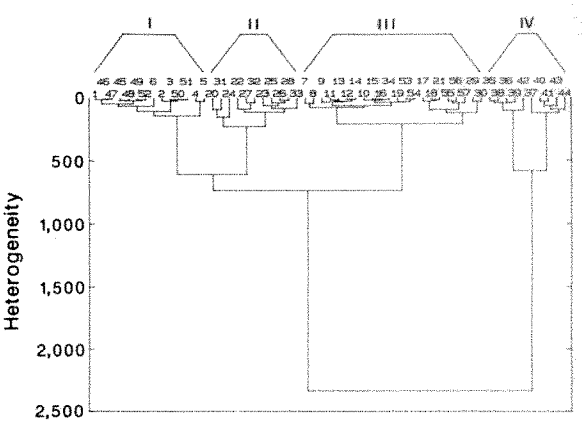

FIG. 2. Dendrogram of the FT-IR classification of a selected series of *Pseudomonas* reference strains (American Type Culture Collection, Rockville, Maryland). I, *Pseudomonas aeruginosa*, II and III, *Pseudomonas fluorescens*, IV, *Pseudomonas cepacia*.

with the aid of comprehensive, computer-stored reference data bases using search algorithms, cluster analysis and multivariate statistics[1-4]. Data evaluation is performed either by a library search, or by a comparison of all spectra collected from identical experimental set-ups. Results are presented by hit lists, dendrograms or by 'factorial maps' displaying graphically the spectral similarities and classifications of the strains under study (see Fig. 2).

The data base of bacterial FT-IR reference patterns that is available in our laboratory comprises spectra from more than 300 very diverse Gram-positive and Gram-negative bacteria. We intend to validate, optimize, and extend the data base and software in the course of the forthcoming joint pilot study. Because of the inherent advantages of FT-IR, it is possible to store data over long periods of time, carry out electronic data transfer to different loci and construct 'home-made' libraries for special user-oriented identifications of unknown species.

Scope of applications

The FT-IR technique is particularly useful for researchers who are engaged in the field of quality and environmental control, as it provides considerable screening power for microorganisms from very diverse human and environmental habitats. It may have application in the recognition of human pathogens in cases where conventional techniques are cumbersome, laborious, inadequate and/or time-consuming, or for which established and routine tests are not yet available. The technology may also help to clarify taxonomic relationships within poorly classified taxa[3].

Microbiological FT-IR may also find application in the tracing of epidemics, when used in conjunction with conventional procedures. The capacity for large-scale identity testing may be useful in the maintenance of strain collections or in the supervision of stock cultures to prevent, for example, subtle changes in the production strains used in industry. FT-IR spectroscopy allows the detection of phenotypic consequences of genetic alterations or the analysis of complex, multi-parameter controlled phenomena, such as the production of the bacterial storage material poly-β-hydroxybutyric acid *in situ*[3].

The FT-IR approach to sleuthing out the identity of a microorganism was initially developed with bacteria in mind. However, the experimental strategies and the special software packages can easily be extended to the characterization of other microorganisms such as viruses, fungi, yeasts, amoebae, and even mammalian cells.

When applying standard FT-IR techniques, the biomass requirements have been scaled down so that single colonies can be taken from the primary culture plates. Using a special light microscope in conjunction with FT-IR, even microcolony spots of less than 40 μm in diameter (which corresponds to less than 10^4 bacterial cells) can be analysed. This approach combines, for the first time, the advantages of light microscopy with the sensitivity and selectivity of FT-IR in a single instrument. Microcolony spots that are suitable for FT-IR microscopy are obtained by using an IR-transparent plate as a stamp to transfer the various locally separated microcolonies from the culture plate onto the IR-sample holder. This replica technique permits the detection and enumeration of different colony spots, even from mixed cultures. Optical properties (colony size, colour, different shapes of microorganisms or different colony organizations) are accessible, operator-controlled or by image processing in the computer. As one can easily obtain spectra from 'what you see', the FT-IR pattern technique is used to differentiate and classify individual flora on a primary culture plate.

The software packages used for microbiological characterizations permit the incorporation of additional discriminative, non-spectral characters, such as morphological data from light microscopy or gas chromatography analysis of whole-cell fatty acid composition. The number of spectral traits can be augmented by data that is accessible from the very same FT-IR instrument. The so-called near-infrared and far-infrared regions and, more recently, FT-Raman spectroscopy can provide a substantial amount of additional and complementary information for dedicated microbiological characterizations.

Any FT-IR instrument can be used for the sensitive and specific monitoring of the various gases (for example, CO_2) that are produced by microorganisms grown in culture. The extra- monitoring capacity of FT-IR can be used to test early bacterial growth in liquid cultures with a sensitivity that is comparable to radioactive isotope tracing[5].

The increasing use of FT-IR spectroscopy in quality control, microbiological screening, environmental and medical control, and basic microbiological research demonstrates that this technique is a versatile research tool for classifying and identifying microbiological samples. □

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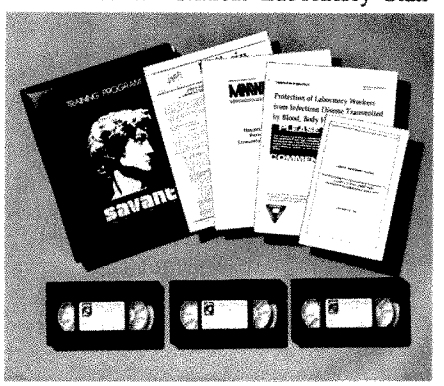
Microbial matters

A monoclonal antibody to *Heliobacter* spp./*Campylobacter* spp., colony counting systems and a pocket-sized microscope for on-the-spot analysis — product news for the microbiologist.

SAVANT, a specialist in audio-visual teaching aids, has introduced a series of programmes entitled *Protect Yourself from AIDS (Reader Service No. 101). These* **audio-visual aids**, aimed at laboratory researchers who are at risk from exposure to infected blood and other body fluids, follow safety recommendations laid down by the Centers for Disease Control, the Occupational Safety and Health Administration and the National Committee for Clinical Laboratory Standards. The video tape, *Protecting Yourself from AIDS: What Everyone Needs to Know* outlines the ways in which HIV is and is not transmitted, together with the causes and symptoms of AIDS. In addition, it reviews the nature and reliability of clinical detection tests for HIV. A second tape, *Protecting Yourself from AIDS: Precautions for Laboratory Workers*, covers safety considerations concerning pipetting, centrifuging and the operation of laboratory equipment, the safe disposal of needles and other sharp instruments, and the clean up and disposal of contaminated wastes.



Audio-visual AIDS.

Reagent round up

Helicobacter spp. and *Campylobacter* spp. can now be easily identified in gastric biopsies using the **monoclonal antibody** that is now available from Bioproducts for Science *(Reader Service No. 102)*. Data indicate that the monoclonal can be used in the immunocytochemical identification of *Helicobacter pylori* in formalin-fixed gastric biopsies. The monoclonal antibody positively labelled *Helicobacter* spp. in 62.4 per cent of the tis-

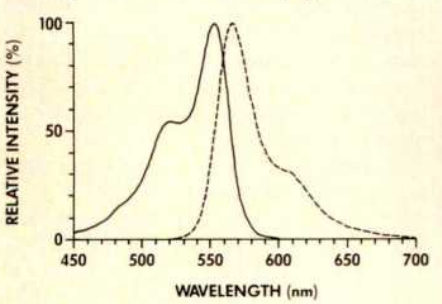
sues from patients with gastritis, says the company. The monoclonal can be used in formalin-fixed, paraffin-embedded tissue as well as cryostat sections. It reacts with the flagellar antigen of most *Helicobacter/Campylobacter* spp.; it does not cross react with other bacteria. The monoclonal is available as lyophilized ascites and is reconstituted to 1.0 ml with sterile distilled water. In addition, a purified FITC-conjugated (fluorescein isothiocyanate) antibody is also available from BPS.

Kirkegaard & Perry has a new **substrate for immunohistochemical staining**, diaminobenzidine (DAB), which produces a sharply localized brown deposit at the reaction site and which is supplied as part of the DAB Reagent Set *(Reader Service No. 103)*. The set includes the DAB substrate concentrate in stable liquid form, buffer concentrate

Diaminobenzidine substrate in kit form.

and hydrogen peroxide solutions. The solutions are provided in dropper bottles that are equipped with controlled-volume tips for ease of use. The need to weigh or handle DAB, a suspected carcinogen, is eliminated as the reagent solution is prepared by dropwise addition, says the company.

Cy3, a cyanine dye developed by Alan S. Waggoner at Carnegie Mellon University and manufactured by Biological Detection Systems, is the first in a new series of fluorophores to be introduced by Jackson ImmunoResearch *(Reader Service No. 104)*. The **fluorophore** is available conjugated to over



Cy3 fluorophore for sale.

270 AffiniPure and AffiniSorbed secondary antibodies, over 60 ChromPure immunoglobulins and their fragments, and streptavidin and avidin. Cy3 is brighter than most other fluorophores, more photostable than fluorescein isothiocyanate, and produces less background than any rhodamine, says Jackson ImmunoResearch.

Frozen/unfrozen assets

Savant Instruments has introduced a **microprocessor-controlled cryopreservation system**, the CPS100, which is designed to increase the viability of stored cell and tissue samples by controlled-rate freezing without the use of liquid nitrogen *(Reader Service No. 105)*. The compact CPS100 unit has a rectangular freezing chamber that measures 15.2 cm × 8.9 cm × 20.3 cm. The two-stage mechanical refrigeration system freezes samples at a selectable rate between 0.1 °C min^{-1} and 9.9 °C min^{-1} from 40 °C down to −60 °C, automatically compensating for the heat of fusion as samples change from liquid to solid. Sample temperatures can be monitored continuously by an RTD probe, or thermocouple. Sample racks are available for holding small ampoules, screw-cap vials and straws. The CPS100 can be preprogrammed with up to 20 run protocols.

To complement Pafra's original formula U-Cool 'undercooling' fluid for the sub-zero temperature storage of aqueous solutions, the company has launched U-Cool 'type E' for the **low temperature storage of labile biomolecules** *(Reader Service No. 106)*. Undercooling works as follows: when dispersed in the U-Cool fluid and cooled to −20 °C, aqueous solutions enter a stability window. Even at −25 °C the sample will not freeze, says Pafra. Consequently, undercooling provides all the benefits of low temperature storage, while avoiding harmful freezing. U-Cool 'type E', which contains an emulsifying additive to aid dispersal of aqueous sample in the inert undercooling fluid, is particularly suitable for the storage of enzymes without loss of activity, says Pafra. Costing £65 (UK) per litre, U-Cool and U-Cool 'type E' can be used for the long-term storage of purified biomolecules, purification intermediates, subcellular fractions or microbial cell cultures.

Colony counting

The Mini Light Box from Manostat is intended as a handy **laboratory light source** for screening multiwell plates, Petri dishes,

Manostat's mini light box.

electrophoresis gels and slides *(Reader Service No. 107)*. The $79 (US) battery-operated light box features a colour-corrected fluorescent light that is evenly diffused over the 4 × 5-inch viewing area. A 6 V d.c. adaptor socket is provided for stationary use of the light box. An optional $79 (US) 1.7× magnification Mini Magnifier is available to assist in the counting of bacterial colonies and hybridoma screening. Removable counting grids, which cost $10 (US) for a pack of five, provide a useful reference when counting colonies. Manostat offers a complete colony counting system, which consists of the Mini Light Box, Mini Magnifier, and colony counting pen, for $300 (US).

Domino, the new **colony counting system** from Perceptive Instruments, is designed to provide accurate plate counts in seconds and has comprehensive data handling facilities *(Reader Service No. 108)*. The system, which can be fully integrated with IBM AT-compatible computers, comprises the image analysis hardware, a high-performance video camera and the Borland Quattro Pro 2 spreadsheet program. Other design features include a modular camera stand, which provides a choice of illumination for counting colonies on different culture media, and a zoom lens attachment for laboratories using different sized Petri dishes or those requiring higher magnifications. Other features include special algorithms to separate touching colonies and mouse-controlled frames to isolate spreaders. The count mode is useful for rough-edged colonies that are often found in settle plates or mammalian cell culture. Domino can also differentiate colonies on the basis of size. Other applications of Domino include antibiotic assays, epi-fluorescence microscopy, multipoint sensitivity testing and toxicological assays.

Mastascan Series 600 is a new **automated reading and reporting system** from Mast Diagnostics for bacterial antibiotic susceptibility and identification tests *(Reader Service No. 109)*. The system permits the microbiologist to analyse multipoint inoculated susceptibility and identification plates with great speed and accuracy, says Mast. Different applications — breakpoints, automatic identifications, M.I.C. determinations and epidemiological analysis — are selected by means of menu displays. The hardware consists of a multipoint inoculator, which is designed for the delivery of 19, 36 or 96 standardized bacterial inocula in a rapid and precise manner, a scanner module (high-resolution CCD colour TV camera), a microcomputer and colour monitor.

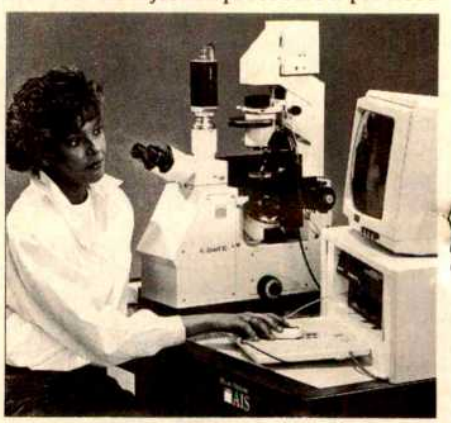
Automate DNA sequence reading and other laboratory tasks with the SciScan 5000 **automated scanning system** from United States Biochemical *(Reader Service No. 110)*. The high-resolution scanner digitally records an image of any laboratory sample, including wet gels and culture dishes, with a scanning bed that accommodates samples of any length, up to 14 inches wide and 0.5 inches thick. USB's BioAnalysis software controls the scanner, displays the scanned images on the computer screen in 'windows', and analyses the images. Analysis can be simple (densitometry and graphing) or complex (DNA sequence reading). The software

USB's automated scanning system scans films, wet gels and culture dishes.

can perform whole-band densitometry with automatic or manual lane and band finding. Stored, digital images of laboratory samples can be modified to enhance contrast, remove background, soften, sharpen or even 'shadow' an image. Each of these functions allow the user to view important aspects in an image while minimizing background. Images can be printed or saved in a standard file format for processing by other programs.

Under the microscope

A new automated system for **microinjection into living cells** has been developed by Carl Zeiss *(Reader Service No. 111)*. The AIS (automated microinjection system), which is based on the new Zeiss Axiovert inverted microscopes, is designed to provide computer-controlled injections into as many as 2,000 cells per hour. The long working distance ICS optics and condensers of these microscopes provide ample space above the specimen for injection tools, says Zeiss. Control over the injection procedure is provided



Computer-controlled microinjection.

by the Microscope System Processor, which features both hard and floppy disk drives and VDU graphic controller. Zeiss says the program controls the positioning of the scanning stage in both the X and Y axis with submicron reproducibility, together with the motorized movement of the micromanipulator in the Z axis, and the CCD camera. The position of single cells can be selected and marked using a menu-selected graphic overlay program, which stores the relevant XY coordinates for rapid retrieval.

The Lensman **pocket-sized microscope**, which is distributed by Inlet, measures just 105 mm (diameter) × 27 mm (thickness) and can be used to perform microscopic examinations anyplace, anytime *(Reader Service No. 112)*. The top surface forms the specimen stage, while the operating controls are placed on the underside. The nine-element folded optical system provides magnifications of 80× and 200× and a resolving power of 4 μm. Slides or opaque objects are placed on top of the Lensman, allowing the light soure, which can be set at high or low power, to illuminate the specimen. Part of the top case articulates to form a lighting arm with a range of illuminations: reflected, dark-ground and transmission. As an optional extra, the Lensman can be fitted with a Photo-adaptor, which allows direct coupling to a 35-mm SLR camera. □

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SCOTGEN

BIOTECHNOLOGY RESEARCH

Scotgen is a leading innovative Biotechnology Research and Development company specialising in Antibody Engineering and DNA Probe technologies. In particular, the company is a world leader in humanising monoclonal antibodies for therapy. We have openings for high calibre PhD Team Leaders and Research Assistants with a B.Sc./M.I.Biol. or equivalent. At least two years' experience in recombinant DNA is preferred for each position. The successful candidates will initially work on the development and application of antibody engineering technologies particularly for pharmaceutical applications. There is considerable scope to contribute to other projects within the company and to influence technical strategy.

Scotgen's laboratories are located within Aberdeen University to the north of the city, 1 mile from open countryside, with a large choice of low-cost housing. With its mild climate and high quality lifestyle, Aberdeen is a major growth area in the UK. We offer competitive salary and a relocation package along with the opportunity for early involvement in an expanding company.

Please send your CV and details of three referees to: The Research Director, Scotgen Limited, 2 Tillydrone Avenue, Aberdeen, AB9 2TN, Scotland.

Closing date: May 24th 1991. (6167)A

PROTEIN X-RAY CRYSTALLOGRAPHER

National Institute of Allergy and Infectious Diseases

National Institutes of Health

A protein crystallography laboratory is being established with intramural AIDS funding to conduct an original research program and to interact with other scientists within the NIH intramural program. Applicants for director of the crystallography lab are being sought. Ph.D. or M.D. applicants should have a record of achievement in solving protein structures by X-ray crystallography. Salary and research resources will be commensurate with credentials and research experience.

Applicants should send curriculum vitae and names and addresses of at least three references to:

John E. Coligan, Ph.D.
Chairman, Search Committee
BRB, NIAID, NIH
Building 4, Room 413
9000 Rockville Pike
Bethesda, MD 20892
Telephone: 301-496-8247
Facsimile: 301-402-0284

NIH is an Equal Opportunity Employer

(NW6668)A

HEAD, DIVISION OF EXPERIMENTAL PATHOLOGY

DEPARTMENT OF PATHOLOGY

MEMORIAL SLOAN-KETTERING CANCER CENTER

Memorial Sloan-Kettering Cancer Center is seeking an individual to head the newly established Division of Experimental Pathology, reporting to the Chairman of Department of Pathology. He/she will be responsible for the overall development of various laboratory programs dealing with the biology of human tumors, as well as programs to facilities and maximumize the transfer of biological findings into diagnostic, prognostic and therapeutic applications. This Division will have close interaction with anatomic pathology and will relate to other clinical and basic science departments in the Center.

The candidate for this position should possess an M.D. and/or a Ph.D., be an internationally recognized figure in research, and have proven ability as an academic leader. Background in the anatomic pathology of human cancer is desirable.

Interested applicants should send their curriculum vitae and bibliography and a brief description of research goals to:

Joseph R. Bertino, M.D.
Chairman, Search Committee,
Memorial Sloan-Kettering Cancer Center,
1275 York Avenue,
New York, New York 10021

An Equal Opportunity/Affirmative Action Employer.

(NW6675)A

National Institutes of Health
Public Health Service
Department of Health and Human Services

CHIEF
Laboratory of Molecular Biology

The National Institute of Neurological Disorders and Stroke (NINDS) invites nominations and applications for the position of Chief, Laboratory of Molecular Biology (LMB), Division of Intramural Research (DIR), Basic Neurosciences Program (BNP). This is a Civil Service Position in the Senior Executive Service with a salary range of $87,000 - $100,500 per annum. Physicians are also eligible for payment of Physicians Comparability Allowance up to $20,000 per year. A one-year probationary period must be served by the individual selected if not currently in the Senior Executive Service.

The Chief, Laboratory of Molecular Biology provides technical and administrative direction and leadership to the Institute's research program in molecular neurosciences and serves as the focal point within the Federal Sector, scientific community and the public for these activities.

For more information on the position, contact Dr. Harold Gainer at (301) 496-5468. For information regarding qualifications requirements, contact Ms. Claudia Palumbo at (301) 496-6334. The following forms are *required*: Application for Federal Employment (SF-171) accompanied by a current curriculum vitae and bibliography. These must be submitted to:

Ms. Claudia Palumbo
National Institutes of Health
NINDS Personnel Office
Building 31, Room 8A23
9000 Rockville Pike
Bethesda, Maryland 20892

Applications must be postmarked no later than September 9, 1991

All qualified candidates will receive consideration without regard to race, religion, color, sex, age, national origin, lawful political affiliation, marital status, union membership, or any nondisqualifying physical or mental handicap.

NIH is an Equal Opportunity Employer

(NW6663)A

HEAD OF MRC/SERC BIOLOGY SUPPORT LABORATORY

The Medical Research Council and the Science and Engineering Research Council pursue a joint programme of research in the biological and biomedical sciences using the Synchrotron Radiation Source (SRS) at the SERC's Daresbury Laboratory. Within this programme the Councils jointly operate a Biology Support Laboratory (BSL) at the SRS to provide the specialist support needed locally by visiting scientists undertaking biological and biomedical projects. It is supported by the service divisions at Daresbury.

Synchrotron Radiation is an important tool in biological and biomedical research which requires continuous vigorous development. The staff of the BSL have an essential role in fostering this development.

Applications are invited for the position of Head of the BSL. The person appointed will have an established reputation as a scientific investigator, an interest in instrumentation, and the ability to manage and co-ordinate the service and research activities. The Head of the BSL will be able to carry out his/her own research, which preferably should be in the field of protein crystallography, and will direct a small number of in-house scientists with whom he/she will be involved in the design and development as well as the operation and maintenance of instruments and facilities provided at the SRS for biological research. Much of the work will be in collaboration with outside users.

The appointment as Head of the BSL will be for a fixed term of up to 5 years in the first instance. The Councils recognise that they are seeking a demanding combination of qualities and experience and are therefore willing to consider a range of options to fill the post. Secondment arrangements between the Daresbury Laboratory and a University or other organisation would be welcomed.

Salary and other conditions of service will depend on the detailed arrangements. Some assistance with expenses for house sale and purchase may be available.

Applications in the form of a short statement (not exceeding 500 words) outlining in general terms the scientific interests and achievements of the applicant, together with a curriculum vitae, list of publications and the names of two referees should be submitted not later than 30th May, 1991 to:
Professor J Bordas, Science and Engineering Research Council, Daresbury Laboratory, Daresbury, Warrington WA4 4AD. Tel: 0925 603507.

For further information about this post please contact Professor J Bordas.

We are an equal opportunities employer. (6165)A

Daresbury SCIENCE & ENGINEERING RESEARCH COUNCIL

UNIVERSITY OF MANCHESTER
SCHOOL OF BIOLOGICAL SCIENCES

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

Applications are invited for a

POSTDOCTORAL RESEARCH ASSOCIATE

The project, funded by a 3 year grant from the Wellcome Trust to Dr R. P. Boot-Handford, Dr K. E. Kadler and Professor M. E. Grant, is to examine the consequences of mutations in the C-terminal domain of the collagen X (ten) gene and in particular how these affect the folding of the translated product. These studies will test a novel model for how collagen chains associate into trimeric complexes prior to triple helix folding and represent an integral part of our research programme to determine the molecular pathology of human collagen X in diseases such as the chondrodysplasias and osteoarthritis. The techniques will involve site-directed mutagenesis, expression of CDNA constructs in mammalian cells and biochemical analysis of labelled protein products. Experience in some aspect of protein analysis and/or molecular biology would be advantageous but is not essential since training can be provided.

This position is available immediately, salary range £11,399-£14,744 p.a., and will attract an additional Wellcome Trust salary enchancement premium of 2 points.

Applications should include a full CV, the names and addresses of 2 referees and should be sent to Dr R. P. Boot-Handford, Department of Biochemistry & Molecular Biology, the University, Manchester M13 9PT, UK (Tel: 061 275 5097, FAX: 061 275 5082) as soon as possible.

The University is an Equal Opportunity Employer. (6141)A

POSTDOCTORAL POSITIONS IN NEUROBIOLOGY

Studies of nicotinic acetylcholine receptors of muscles and nerves and the autoimmune response to nicotinic receptors of muscle require candidates with MD or PhD degrees experienced with either **molecular genetics** (for cDNA cloning, mutagenesis, and expression), or experienced with **protein biochemistry and monoclonal antibodies** (for receptor purification and characterization, and monoclonal antibody production, and studies of subunit synthesis and assembly), experienced with **electrophysiology** (for studies of receptors expressed from cDNAs in *Xenopus* oocytes and transformed cells), or experienced with **histology** (for immunohistological localization of subunit proteins and in situ hybridization localization of subunit mRNAs). For the right candidates, there is opportunity to work in several of these areas in a highly productive lab.

Send your curriculum vitae and names, address, and telephone numbers of three references to:
Dr. Jon Lindstrom, Institute of Neurological Sciences, University of Pennsyvania School of Medicine, 215 Medical Education Bldg., Philadelphia, PA 19104-6074. Fax (215) 573-2015.

(NW6676)A

UNIVERSITY COLLEGE LONDON

Medical Molecular Biology Unit

Molecular Virology

GRADE D TECHNICIAN

Applications are invited for the above position (supported by Birthright) to work with Dr D. S. Latchman on a project studying the role of cellular transcription factors in regulating human papillomavirus gene expression in normal and malignant cervical cells.

Previous experience in molecular biology is desirable but not essential as training can be provided. The position is for three years and is available immediately or by arrangement. Salary is on the TD scale up to £10,924 plus £1,873 London Allowance.

Applications (with CV and names of two referees) to Dr D S Latchman, Medical Molecular Biology Unit, Department of Biochemistry & Molecular Biology, Windeyer Building, Cleveland Street, London W1P 6DB.

Equal opportunities employer.

(6119)A

MOGEN International nv is a plant biotechnology company located at the Bio Science Park near Leiden University in The Netherlands and active in genetic engineering of agricultural and horticultural crops. Within a multidisciplinary research team on the development of fungal resistant transgenic plants the following positions are open:

MOGEN
The agricultural bio-engineers

protein biochemist

who will collaborate in a project aimed at the development of fungal resistant plants. The research in concentrated on the purification and characterisation of proteins with an anti-fungal activity. The eligible candidate has several years of experience with protein purification techniques. Experience with plants is not required.

plant pathologist

The scientist will work in a project aimed at the development of fungal resistant plants. Responsibilities include the development of fungal resistance tests. The eligible candidate has a MSc or Ph.D in phytopathology and several years experience with greenhouse and field plot research techniques. Knowledge of statistical analysis of research results is an asset.

Appointment will take place on the basis of a four-year contract. We offer good conditions, a stimulating scientific environment and a well-equipped laboratory.

Applications with curriculum vitae and referees should be sent to Judith Van Veen-Vrolijk, Einsteinweg 97, 2333 CB Leiden, The Netherlands. For more information please contact Dr. Ben Cornelissen or Dr. Leo Melchers (phone: +31 - 71 - 258282).

(W8447)A

MARION MERRELL DOW

Strasbourg Research Center

invites applications for a position of

Protein X-Ray crystallographer

The Marion Merrell Dow Research Institute is expanding its Biophysics Group to include X-ray crystallography. Our multi-disciplinary team employs a wide range of experimental techniques such as 500 MHz NMR, fluorescence, FT-IR, Raman and CD spectroscopy and molecular modelling in the determination of three-dimensional structures for use in drug discovery.

This commitment has created the need for an experienced protein crystallographer. The ideal candidate will have post-doctoral experience and be familiar with data collection, structure solution and refinement and modelling. Computing facilities include graphics work stations (Silicon Graphics IRIS, Evans & Sutherland PS390) and a Local Area Network with local VAX/VMS and UNIX computers and remote access to the company's CRAY supercomputer.

Our Research Center is located in Strasbourg, France, the crossroads of Europe. With its excellent University, beautiful Cathedral and lovely setting on the Rhine, Strasbourg is a particularly inviting place to live.

Please send CV + 3 names of referees to M.J. Bildstein, Human Resources Department, Marion Merrell Dow Research Institute, BP 447 R/9, 16 rue d'Ankara, 67009 Strasbourg Cedex.

dkfz

Am Deutschen Krebsforschungszentrum ist eine

Professur (C4)

für

Toxikologie

in Verbindung mit der *Fakulät für Theoretische Medizin der Universität Heidelberg* zu besetzen.

Mit dieser Stelle verbunden ist die Leitung einer Abteilung am Deutschen Krebsforschungszentrum.

Bewerber/innen sollten durch herausragende wissenschaftliche Arbeiten in der chemischen Carcinogenese und/oder experimentellen Chemotherapie ausgewiesen sein. Der Schwerpunkt der Aufgaben liegt in der Forschung; das Fach sollte in seiner ganzen Breite vertreten werden können.

Bewerbungen mit den üblichen Unterlagen sind bis zum 23, Juni 1991 an den Stiftungsvorstand des Deutschen Krebsforschungszentrums (DKFZ), Im Neuenheimer Feld 280, D-6900 Heidelberg 1 zu richten.

(W8440)A

UCL

University College and Middlesex School of Medicine

UCMSM

Chair of Medicine

Applications are invited for an established Chair of Medicine and Headship of the Academic Division of the Department of Medicine at the Whittington Hospital. The Whittington, Middlesex and University College Hospitals, are major teaching hospitals of the School of Medicine. The appointee will be a member of the Board of Medicine, which includes Professors and Readers from the Departments of Medicine, Clinical Pharmacology, Nuclear Medicine, Oncology, Haematology, Genitourinary Medicine, Neurology, Nephrology, and Geriatrics. Applicants should have a strong commitment to undergraduate and postgraduate teaching and a personal record of distinction in research. No specific specialty interest is sought, but due consideration will be given as to how the interests of candidates relate to academic and clinical activities at the Whittington Hospital. The appointee's research laboratories will be located at the Whittington Hospital with an optional extension in the Rayne Institute which houses the research facilities of members of the Department of Medicine.

Applications, including a full curriculum vitae (10 copies, one for overseas candidates) and the names of 3 referees should be made within 4 weeks of the appearance of this advertisement to the Provost, University College London, Gower Street, London WC1E 6BT. Further particulars about the post are available from the Dean, University College and Middlesex School of Medicine, Riding House Street, London W1P 7PN (telephone 071-380 9372). Equal opportunities employer.

(6136)A

Human Cancer Genetics Research Unit

Two Post-Doctoral Research Associates

Applications are invited for two post-doctoral research associates to join a new cancer research unit now being set up at Brunel to study the genetic basis of rate-limiting stages in clonal evolution and malignant progression.

One post, funded by the **Cancer Research Campaign (CRC)** is for a **eukaryotic molecular geneticist**. Work will involve the use of retroviral insertion mutagenesis techniques to identify and clone genes which control proliferative capacity in rodent and human cells. Somatic cell genetics skills, such as microcell fusion and fluorescence *in situ* hybridization, would confer an advantage but are not mandatory. There is, however, a requirement for experience in gene manipulation, preferably involving mammalian gene cloning.

The second post, supported by the **United Kingdom Co-ordinating Committee on Cancer Research (UKCCCR)** is for a **mammalian cell biologist** to conduct a quantitative study of the effects of low-dose ionizing radiation on induction of stages in malignant transformation. Initially, emphasis will be placed on induction of the 'Infinite Self-Renewal' (ISR) phenotype, which is a prerequisite for clonal evolution and for which well characterized clonogenic cell culture assays have already been developed (see *Nature* **299**, 633 and *Natu* **304**, 648). Applications for this position should have a solid background in quantitative mammalian cell culture techniques, preferably involving *in vitro* mutagenesis or transformation systems.

Further information concerning both projects can be obtained from Professor R F Newbold (Tel 0895 74000 Ext 2090).

Both positions are for a period of three years in the first instance. Salary is within the University RA 1A scale, up to £15,805 per annum including London Weighting. Application forms can be obtained from the Personnel Office, Brunel University, Uxbridge, Middlesex, UB8 3PH, or by telephone on 0895 812304 (24 hours) quoting post references: Q6931 for the CRC position and RAD90/6 for the UKCCCR vacancy. Closing date: 10 June 1991.

(6128)A

Brunel

THE UNIVERSITY OF WEST LONDON

ENDOWED CHAIR IN ORNITHOLOGY

James Ford Bell Museum of Natural History

UNIVERSITY OF MINNESOTA

The University of Minnesota, Twin Cities Campus, invites applications and nominations of distinguished scientists for the newly endowed Breckenridge chair in Orthnithology at the rank of tentured Associate or Full Professor. Qualifications include: an outstanding record of scholarly achievement, a strong record of extramural support, and a vigorous research program in ornithology. The Bell Museum is commited to the establishment of strong research and teaching programs with a focus on the most recent advances in comparative avian biology and conservation. The successful candidate will be expected to conduct a vigorous research program, to teach, and to contribute to the Museum's public programs. The endowment was established through broad public support and the person selected will have high visibility in the community. Tenure will be in one of the academic departments of the College of Biological Sciences, most probably Ecology, Evolution, and Behavior.

Applications should include curriculum vitae, reprints, a statement of research interests, and names of at least three references and be sent to: Breckenridge Chair Search Committee, Bell Museum of Natural History, University of Minnesota, 10 Church St. S. E., Minneapolis, MN 55455. Applications will be accepted until 1 October 1991.

The University of Minnesota is an equal opportunity educator and employer.

(NW6671)A

Key Management Roles Within A Major New Research Centre

International Research in Madrid, Spain

SmithKline Beecham is a major force within the international pharmaceutical industry and enjoys a substantial track record of discovering and developing new medicines. Our vast commitment to research and development is seen as a high priority within the company and is conducted at several research centres around the world.

Currently, we are creating a new research centre at the Technology Park at Tres Cantos in Madrid, Spain. This centre, when fully operational, will make a major contribution to our international research programme. To help achieve this, we need the following high-calibre individuals.

Associate Director, Biotechnology

A significant proportion of the research to be undertaken at the centre will involve a fundamental programme on gene expression in micro-organisms, in particular, Streptomyces. As such, we are looking for a scientist (4 years plus post PhD) with extensive experience in biotechnology to manage a section working on microbial genetics and molecular biology. Reporting to the overall director of the research centre, you will also liaise with the company's Biopharmaceuticals R&D group in both the UK and USA.

Research Managers

A major area of research to be undertaken will be natural product screening to identify selected agents with antimicrobial and/or pharmacological activity. Both antimicrobial assays and mechanism-based screens will be used. We are now looking for three experienced scientists (3 years plus post PhD) to report directly to the overall research centre director and be responsible for managing sections involved in the following activities: microbiology, molecular-targetted screening, and natural product chemistry.

For all the above positions, not only will you need to have excellent scientific ability, but you must also have strong organisational and managerial skills and, moreover, a good command of Spanish and English.

As you would expect from a company of our stature, we offer a first-class salary and benefits package which includes generous relocation assistance to this historic part of Spain.

To apply, please send full career details, indicating which position you are interested in, to SmithKline Beecham Pharmaceuticals, Division de Personal, Apdo. de Correos no. 36137, 38080 Madrid, Espana.

SB
SmithKline Beecham
Pharmaceuticals

(6155)A

DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF OXFORD

MICROBIOLOGY UNIT

Postdoctoral Research Assistant (RS-1A) BR/362

A postdoctoral position is now available for a yeast molecular geneticist or molecular biologist, to work with Professor Paul Nurse and Dr Peter Parker, on genetic analysis of the phosphatidyl inositol-3-OH kinase pathway in fission yeast and mammalian cells. The post, which is British Heart Foundation funded, is available for up to 3 years.

Salary on the scale £11,399-£18,165 per annum.

Applications, including a full curriculum vitae, and the names and addresses of two referees should be sent to:
The Senior Administrator

An Equal Opportunity Employer. (6160)A

DEPT OF BIOCHEMISTRY, SOUTH PARKS ROAD, OXFORD OX1 3QU

UNIVERSITÄT ULM - KLINIKUM -

Research scientist and Post-doc

position available at the Department for Immunology of the University of Ulm. The department's interest focuses on: Immune response to intracellular bacteria; heat shock proteins and immunity. We are a multidisciplinary group and candidates with expertise in molecular biology, protein chemistry, or cellular immunology will be considered. Salary according to BAT IIa/C 1 (70,000 - 80,000 DM) depending on family status and age. Send application including list of publications, CV and names of referees to:

Prof. Dr. S. H. E. Kaufmann, Department for Immunology, University of Ulm, Albert-Einstein-Allee 11, W-7900 Ulm, FRG. (W8435)A

Ministry of Agriculture, Nature Management and Fisheries

CENTRE FOR PLANT BREEDING RESEARCH WAGENINGEN, THE NETHERLANDS

The Centre for Plant Breeding Research CPO aims to contribute to a higher quality of Dutch and European agriculture by innovating plant breeding methodology. The institute employs more than 100 scientists working in fields such as plant molecular biology, cell biology, quantitative genetics, biochemistry and phytopathology.

Vacancy:

senior research scientist (f/m)

Nematology

General:
CPO has an opening for an enthusiastic committed scientist who will cary out research on resistance against nematodes in potato, in particular the potato cyst nematode. Quantitative genetics, marker-aided selection, immunochemistry and histological studies are key words describing the research work. A well equipped laboratory and modern quarantine glasshouse facilities are available.

Duties:
you will be part of a multi-disciplinary team of 4 senior scientists, 2 junior scientists and 6 technicians working on the genetic improvement of potato. You will also be asked to act as an adviser for other CPO projects studying resistance against nematodes.

Qualification:
ideally you will be qualified to PhD or equivalent level in nematology. Knowledge of plant genetics and plant breeding is very desirable.

You will be expected to obtain at least a working knowledge of the Dutch language, although in the laboratory English can be used for cummunication with your colleagues.

Salary:
initial salary within the range DFL 58.000 - DFL 73.000.

Initially you will be offered the job for a 3 year period. Afterwards tenure is a possibility. This job could also be carried out on a part-time arrangement. CPO is an equal opportunity employer.

Informal enquiries to Dr J. Hoogendoorn (31 - 8370 - 77012).

Please write, with full details of your qualifications and experience and the names of three referees, quoting ref 2731.081009, to the Personnel Officer, CPO, P.O. Box 16, 6700 AA Wageningen, The Netherlands. Closing date: 15 May 1991.

(W8449)A

NEWCASTLE HEALTH AUTHORITY

NEWCASTLE GENERAL HOSPITAL UNIT

PRINCIPAL CLINICAL SCIENTIST (GRADE B)

REGIONAL IMMUNOLOGY DEPARTMENT — NEWCASTLE GENERAL HOSPITAL

Applicants are invited for the above post which is located in the Regional Immunology Laboratory at Newcastle General Hospital which provides a regional service for patients with immunodeficiency disease (both primary and secondary) autoimmune disease and allergies.

Applicants should hold a good honours degree in immunology, microbiology, biochemistry, molecular biology or a related biology or a related subject. Ideally they should have significant experience in cellular immunology with techniques.

The successful applicant will be expected to undertake a senior role in the department. Current departmental and chest diseases. Applicants may, if appropriate, be able to register for a higher degree.

Salary: £12,644 — £21,895.
For further information/informal visit please contact Dr A Fay on 091 2738811 ext. 22741.
Applications by means of detailed CV giving names and addresses of 2 professional referees to the Personnel Director, Personnel Department, Milvain Building, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, NE4 6BE.

Closing date: 30 May, 1991.

(6151)A

PAISLEY COLLEGE

DEPARTMENT OF BIOLOGY
LECTURER IN PLANT BIOLOGY

Paisley College is a major degree awarding institution funded by the Scottish Education Department with some 3,500 full-time equivalent students on vocationally orientated degree and honours degree courses.

Applications are now invited for the above post. Candidates should be qualified to honours degree level and should have experience and interests in the areas of plant physiology, tissue culture and/or biochemistry. In addition to teaching duties, staff are encouraged to engage in research and consultancy.

The appointment will be made on a two year fixed term basis.

Salary Scale: £11,919-£17,724 (bar) — £21,390 pa (under review)

Application forms and further details may be obtained from the Personnel Office, Paisley College, High Street, Paisley, PA1 2BE (Tel. No. 041 848 3692) to which completed applications should be returned by 23 May, 1991. Informal enquiries may be made to Professor W Stevely, Head of Department on 041 848 3100. (6123)A

Coláiste na hOllscoile Corcaigh
University College Cork

RESEARCH SCIENTISTS IN MICROBIOLOGY

Applications are invited for Research Scientist posts (2) to work in the area of plant-microbe interactions in the Microbiology Department, UCC. The research programme at UCC focuses on both basic and applied aspects of nitrogen fixation in *Rhizobium* spp. and biological control of phytopathogens by *Pseudomonas* spp.

Two vacancies exist for postdoctoral scientists to join our multidisciplinary team to work on (i) molecular biology aspects of gene regulation in *Rhizobium* or *Pseudomonas* (Molecular Biologist/Geneticist); (ii) soil microbiology/ecology aspects of plant-microbe interactions (Soil Microbiologist/Microbial Ecologist).

Applicants for the posts should preferably hold a PhD degree or have equivalent experience. The posts will be initially offered on a one-year renewable contract. Salary will be competitive and commensurate with experience, and it is intended to fill the posts immediately.

Applicants should submit a full CV and arrange for 2 letters of reference to be submitted to: Professor F O'Gara, Microbiology Department, University College, Cork, Ireland. Tel: (+353-21) 276871. Fax: (+353-21) 275934.

University College Cork is an Equal Opportunity Employer. (6163)A

Veterinary Immunologist

TOP RESEARCH RECORD?

$34,434 to $50,749

($40-$64K package)

Australian Animal Health Laboratory
Geelong, Victoria
Australia

A new position for a Veterinary Immunologist has been created at the Australian Animal Health Laboratory and we are seeking a Research Scientist with experience in ruminant immunology, preferably with sheep. This is a key position within an established team investigating the use of live recombinant viral vectors as veterinary vaccines. We expect that the successful applicant will have a top research record, demonstrated, for example, by relevant publications in international journals.

The successful candidate will be required to undertake investigations into the role of cylokines in the recovery of sheep from infectious agents, including bluetongue virus, and to investigate the immunogenicity of native and modified viral antigens expressed from live viral vectors. The appointee will need the ability to initiate and carry out original research, to work effectively in a team and to supervise staff. A PhD or equivalent qualification in a relevant field is required and a Veterinary degree would be an advantage.

The Australian Animal Health Laboratory (AAHL) is a high microbiological security laboratory involved in the diagnosis and prevention of animal diseases exotic to Australia. Its research programs aim to enhance Australia's preparedness to combat exotic diseases of livestock species by the development of rapid diagnostic capabilities. The Laboratory is recognised as the most sophisticated of its type and has excellent facilities for immunology, molecular virology, histopathology and electron microscopy.

Geelong offers an enjoyable lifestyle. It is within 50 minutes easy drive to Melbourne and has the advantages of a major city in a semi-rural environment. Geelong has excellent educational facilities, including a University and recreational facilities including ocean beaches within 20 minutes from the Laboratory.

Dr Marion Andrew can provide more details of the position. A job description, selection criteria and a copy of the microbiological security conditions which pertain to the position can be obtained by phoning the receptionist on (052) 26 5222 or alternatively by fax on (052) 23 1424.

Please send your application including details of your skills, qualifications and work achievements and the names of at least two professional referees, marked "Confidential", to: **The Head of Laboratory, CSIRO, Australian Animal Health Laboratory, PO Bag 24, Geelong, Victoria 3220, Australia.** Closing date for applications: 10 May 1991. (W8438)A

CSIRO
AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

HDM 9780

UNIVERSITY OF CAMBRIDGE

Department of Anatomy

POST-DOCTORAL SCIENTIST

Applications are invited for an MRC-funded position to work on the molecular basis of homeotic gene function in *Drosophila*. The project will focus on the regulation and function of target genes under the control of the homeotic gene *Ultrabithorax*. The post will initially be funded to the end of Dec. 1992. Salary £11,399 – £16,755. Applications with full CV and names of two referees a.s.a.p. to **Dr. Rob White, Department of Anatomy, University of Cambridge, Downing Street, Cambridge. CB2 3DY. Fax (0223) 333786.** (6156)A

RESEARCH INSTRUCTOR

Non-tenure accruing faculty position is available in Department of Biochemistry to teach and supervise graduate students and postdoctoral fellows and to perform research on molecular mechanisms involved in regulation of surfactant protein gene expression in fetal lung.

PhD or equivalent degree, as well as 2-4 years of postdoctoral research experience in biochemistry-molecular biology is required. Applicant should have knowledge of lung cell biology and surfactant biochemistry. Experience is required in preparation/Screening of cDNA and genomic libraries, DNA sequencing, genomic footprinting and DNA affinity chromatographpy.

Submit CV and brief summary of research experience to: **Dr Carole Mendelson, Department of Biochemistry, UT Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75235.**

An equal opportunity/affirmative action employer. (NW6666)A

University of Sheffield

DEPARTMENT OF MEDICINE

NON-CLINICAL LECTURESHIP

Applicants experienced in Immunology are invited for the post of non-clinical lecturer in the Department of Medicine, Clinical Sciences Centre, Northern General Hospital. This post extends the Department's interest in thyroid autoimmunity, with particular emphasis on immunogenetics and T cell regulation, under the direction of Professor Anthony Weetman. Although not an essential requirement for this position, experience in molecular aspects of Immunology would be helpful.

Applicants will be in immediate charge of a research group, with funding provided by the Wellcome Trust. They must be capable of developing and leading a competitive research group in endocrine autoimmunity and of establishing collaboration with other groups.

Informal enquiries to Professor A. P. Weetman (Tel: 0742) 434343 ext 4908/4601.

Written further particulars from Director of Personnel Services, Personnel Department, PO Box 594, Firth Court, Western Bank, Sheffield S10 2UH (Tel: 0742-768555 ext 4144) to whom applications, including a CV and the names/addresses of three referees (6 copies of all documents) should be sent by 24 May 1991. Please quote Ref: MAP389/G.

An Equal Opportunity Employer. (6134)A

at the leading edge

EXECUTIVE EDITOR

Major New Journal on the Sciences of Complexity

Pergamon Press, together with the Santa Fe Institute, is launching a new journal devoted to the sciences of complexity, and requires a full-time Executive Editor for the day-to-day management of this publication, under the guidance of the academic Editor-in-Chief and an international Advisory Editorial Board. This newly created position presents an exciting opportunity to pursue a career in scientific publishing covering one of the most rapidly developing fields in science.

The Executive Editor will be located at the Santa Fe Institute, in Santa Fe, New Mexico, USA. Created in 1984, the Santa Fe Institute is a private, independent organization dedicated to research into complex systems, ranging from the global economy to the immune system, to the learning process.

Reporting to the Executive Vice President of the Santa Fe Institute and the Editorial Director of Pergamon Press, the Executive Editor will be responsible for the day-to-day management of the journal and for its developlment Duties will include the commissioning and technical editing of articles in liaison with the Editor-in-Chief and the Editorial Board, and keeping abreast of developments in the field. The Executive Editor will be expected to travel to important conferences, and to develop contacts with institutions and research workers in the field of complexity.

Candidates will have at least a first degree, and preferably a higher degree, in any of the natural or social sciences, must have good communication skills, and demonstrable knowledge of complex and adaptive systems. The ability to present technical information in a concise, readable way, an eye for detail, and the ability to work to deadlines and as part of a team are essential. Previous experience in publishing would be an advantage, but all candidates with relevant experience in complex and adaptive systems, whether from a publishing background, or from a technical background, will be considered.

The Santa Fe Institute has a pleasant location in Santa Fe, New Mexico, one of the leading resort communities of the American south west. A competitive remuneration package is available.

Applications, in writing, accompanied by a full Curriculum Vitae, should be sent to: Dr Peter T Shepherd, Editorial Director,

Pergamon Press plc,
Headington Hill Hall,
Oxford OX3 0BW.

(6164)A

PERGAMON

EMBL

The European Molecular Biology Laboratory, an international research organization situated in Heidelberg, Germany, has the following vacancy:

TECHNICAL ASSISTANT

The successful candidate will join a new group in our Scientific Programme "Gene Expression" engaged in research on chromalin structure and gene expression. He/she will participate in the maintenance of a large population of fruit-flies (Drosophila melanogaster) and the biochemical characterization of a variety of activities from fly embryos. The person appointed will be involved in the identification, characterization and purification of protein components required for cell free transcription, replication and chromalin assembly.

Experience in biochemical and molecular biology techniques will be of advantage. The willingness to collaborate in a young team of researchers including students is a prerequisite.

We offer an above-average salary, plus certain allowances depending on personal circumstances.

EMBL
Postfach 10.2209
D-6900 Heidelberg
Federal Republic of Germany

Please write to the Personnel Section for an application form, quoting reference no. 91/21.

(W8446)A

UNIVERSITY OF ALABAMA AT BIRMINGHAM

The Department of Cell Biology in the schools of Medicine and Dentistry at UAB is seeking a molecular biologist to fill a tenure-track position at the level of Assistant, Associate or full Professor. Applicants should have a PhD or equivalent degree, postdoctoral experience and appropriate evidence of research productivity. Candidates using molecular biological approaches to address problems in all areas of cell biology will be considered. Duties will include a modicum of teaching in either histology or neuroanatomy, teaching graduate courses, the training of PhD candidates, and the development of a strong, extramurally funded research program. Salaries and start-up funds are competitive and commensurate with qualifications and experience.

Applications, including the names of three references, a current curriculum vitae, and a summary of research plans should be sent by June 30, 1991 to: Dr Gerald M Fuller, Department of Cell Biology, University of Alabama at Birmingham, UAB Station, Birmingham, AL 35294-0005.

The Univesity of Alabama at Birmingham is an Equal Opportunity/Affirmative Action employer. (NW6670)A

AGRICULTURAL AND FOOD RESEARCH COUNCIL
INSTITUTE OF ANIMAL PHYSIOLOGY AND GENETICS RESEARCH
Babraham, Cambridge CB2 4AT
POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for the above post to work on a project, funded for 4 years by AFRC, to study the molecular biology of cellulases and xylanases using recombinant DNA methodology. The object of this study is to determine the functional significance of the catalytic and non-catalytic regions of these multidomain proteins, and to use the data generated to construct novel enzymes. Although experience in recombinant DNA techniques or protein chemistry would be an advantage, it is not essential. The person appointed will collaborate closely with an AFRC Linked Group based at the University of Newcastle upon Tyne.

The Postdoctoral Research Associate will be paid according to the University Grade 1A Scale, £11,399 – £18,165 per annum, depending on qualifications and experience.

Further particulars can be obtained from Dr G. Hazlewood on Cambridge 832312, ext. 313.

Application forms available from the Personnel Officer, quoting Ref. GH1/ST by 15th May, 1991.

(6116)A

POSTDOCTORAL RESEARCH ASSOCIATE

position is immediately available to work on Gestational Trophoblastic Neoplasms. Experience in culturing cancer cells, regulation of their biochemical and morphological properties by using variety of techniques including molecular biology is desirable. If interested, contact: **Dr. Ch. V. Rao, Dept. of Ob/Gyn., 438 MDR Bldg., University of Louisville, Louisville. KY 40292, Fax (502) 588 0881.**

(NW6677)A

MEDICINAL CHEMISTRY ORGANIC SYNTHESIS

We have successfully completed the first phase of the expansion of our Chemistry Division, having recruited 10 additional chemists including a Head of Immunomodulation – Chemistry. As part of the second phase which will culminate the inauguration of our new chemistry laboratories we are now looking to strengthen the existing team of medicinal chemists by the appointment of a further experienced professional.

TEAM LEADER IMMUNOMODULATION

We seek an outstanding and dynamic individual to lead a team of graduates and PhDs dedicated to the above area. Candidates should be recently qualified to PhD level in organic synthesis with several years postdoctoral experience within the pharmaceutical industry. Preference will be given to those with molecular modelling skills and an understanding of protein structure and enzymology. Experience in the area of immunomodulation, although not essential, would be an advantage.

This position offers a highly competitive salary, supported by a comprehensive benefits package which includes a company car and full relocation allowance. The scope for professional development and promotion is excellent.

Please write, with full details of your qualifications and experience to: The Recruitment Officer, Celltech Ltd., 216 Bath Road, Slough, Berkshire SL1 4EN, UK, quoting ref: 529.

(6166)A

CELLTECH

POSITIONS AVAILABLE IN MOLECULAR BIOPHYSICS

The School of Arts and Sciences of the Homewood Campus of The Johns Hopkins University is expanding its interdepartmental program in Molecular Biophysics spanning the Departments of Biology, Biophysics and Chemistry. Applications are invited for two tenure-track faculty positions in this area of scientific research at the rank of Assistant, Associate or Full Professor, depending on the credentials of the candidate.

We are seeking individuals with expertise in the area of macromolecular structure determination, particularly by high field NMR and single crystal x-ray crystallography. The successful candidates are expected to develop internationally competitive research programs and augment the graduate and undergraduate teaching of the Molecular Biophysics curriculum of this campus. Interested applicants should mail their curriculum vitae, summary of research plans, representative publications and names of three referees to: Sandra Taylor, Search Committee in Molecular Biophysics, School of Arts and Sciences, 102 Macaulay Hall, Homewood Campus, The Johns Hopkins University, Charles and 34th Streets, Baltimore, Maryland 21218.

The Johns Hopkins University is an Equal Opportunity, Affirmative Action Employer.

(NW6672)A

Coláiste na hOllscoile Corcaigh
University College Cork

DEPARTMENT OF FOOD MICROBIOLOGY

Applications are invited for the vacant post of College Lecturer in the Department of Food Microbiology. The Department caters for undergraduate and postgraduate students in both General and Food Microbiology. This post will strengthen the Department's teaching and research programmes and applications from candidates with research experience in industrial aspects of mycology will be particularly welcome.

For informal discussion, contact Professor S. Condon, Tel: [+353-21] 276871, ext. 2396.

Salary scale: IR£14,643-IR£20,138 Bar IR£20,068-IR£25,596 p.a.

Application forms and further details of the post may be obtained from the undersigned. Tel: [+353-21] 276871, ext. 2364. Fax. No. [+353-21] 275948.

Latest date for receipt of completed applications is Friday, 24 May, 1991. M. F. Kelleher, Secretary, University College Cork, Ireland.

University College Cork is an Equal Opportunity Employer. (6130)A

SANDOZ PHARMA LTD.,

a leading international pharmaceutical company based in Switzerland, wishes to appoint a

Pharmacologist/Biologist with experience of animal models and of bone disorders.

The successful candidate will join our established osteology group to supervise the work of a research laboratory commited to the development of animal models of osteoporosis.

Sandoz Pharma Ltd. offers an excellent working environment for scientists, with well-equipped laboratories and opportunities for publishing results and presenting them at international scientific meetings. A "flexitime" system is in operation, allowing considerable personal freedom in the question of working hours.

All applications will be treated with complete confidentiality. Please apply in writing, including a curriculum vitae and a list of publications, to:

Sandoz Pharma Ltd.,
Personnel Department, Ref. 1430
P.O. Box
CH-4002 Basle, Switzerland (W8450)A

SANDOZ

King's College London
Biomolecular Sciences

A Two Year Post-Doctoral Research Position

supported by the AFRC is available immediately to undertake studies of plant membranes and lipids of fatty acid defective mutant strains. The work is varied and would be suitable for applicants with a background in chemistry, physics or biology. Salary on the research 1A Scale, £13,166-£19,932 inclusive.

For further information contact Professor P J Quinn, Biochemistry Section, King's College London, Campden Hill Road, London W8 7AH (tel: 071-333 4408). Applications including two referees, should be sent to Mr D M Drummie at the same address to arrive by 16 May 1991. (6148)A

UNIVERSITY COLLEGE AND MIDDLESEX SCHOOL OF MEDICINE
Department of Molecular Endocrinology
RESEARCH ASSISTANT

Applications are invited from candidates with an honours degree in biochemistry to participate in a research project investigating the influence of the intrauterine thyroid hormone environment on the ontogenesis of brain neurotransmitter and thyroid hormone receptor systems. An interest in molecular biology and neurochemistry would be advantageous. The successful candidate should be able to work independently as well as part of a team. The post is funded by a grant for three years. Starting salary £13,853 pa (incl L.W.). Applications to be submitted to: Dr. A. K. Sinha, Department of Molecular Endocrinology, UCMSM, Mortimer Street, London W1N 8AA, together with a CV and the names and addresses of two referees. Closing date three weeks from the date of advertisement. Equal opportunities employer. (6135)A

University College London
Department of Genetics and Biometry
RESEARCH ASSISTANTSHIP

Applications are invited for a three year Research Assistantship, funded by the Leverhulme Trust, to study the evolution of mate choice, sexual ornamentation and display in Trinidadian Guppies, commencing Aug-Oct 1991. The project involves laboratory experimentation and fieldwork. The successful candidate is expected to register for a PhD. Salary will be on the Grade 1B scale (£11,399-£13,495) with a London weighting (£1,767). The project will be supervised by Dr Andrew Pomiankowski.

Candidates should hold, or expect to gain, a first or upper second class honours degree in the Biological Sciences.

Applications containing CV and the names, addresses and telephone numbers of two referees should be sent to Dr Andrew Pomiankowski, Department of Genetics and Biometry, University College London, Wolfson House, 4 Stephenson Way, London NW1 2HE, Tel: 0865 271218.

Equal opportunities employer. (6120)A

UNIVERSITY OF LIVERPOOL
Department of Biochemistry
POSTDOCTORAL SENIOR RESEARCH ASSISTANT

Initial salary within the range £11,399-£14,744 per annum. Tenable for 1 year 8 months in the first instance, from a date to be arranged.

To work on a project funded by the Cancer Research Campaign to study the regulation, by steriod hormones and by anti-hormones, of specific gene expression in human breast cancer cells in culture. Experience in molecular biology and cell culture techniques an advantage but training can be provided.

Informal enquiries to Dr C D Green on 051 794 4365

Applications by CV with the names, addresses and telephone numbers of three referees, should be received not later than 30 May 1991, by the Director of Staffing Sevices (AS), The Univesity, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote ref. RV/948/N.

An Equal Opportunity Employer. (6132)A

An important career opportunity in Behavioural Pharmacology . . .

Associate Director – Biomedical Research

c. £45,000, plus company car and benefits

Behavioural Pharmacologists . . . if you are ready to direct your own research group . . . then this is the position for you.

My client is one of the world's leading pharmaceutical corporations. Their CNS discovery group in the UK concentrates on anxiety, depression, schizophrenia and related diseases, investigating actions such as 5-HT, GABA and membrane ion channel mechanisms.

As Associate Director of Biomedical Research, you will manage a professional scientific group in neuro- and psycho-pharmacological research. You will provide the focus to stimulate innovation, building the status and visibility of the group to international levels.

The position provides the opportunity to raise your personal standing in the scientific community and develop your own research ideas. You can also develop your career within a pharmaceutical major and, in the medium term, will be a contender for a more senior role.

At least in your mid-30's with a PhD, you are a Behavioural Pharmacologist/Physiological Psychologist of international repute with publications to match. You may be working overseas and wish to use this opportunity to return to the UK.

The company is situated in a pleasant location to the west of London. Full management benefits apply, including a car and generous relocation package.

If you are seeking a new scientific challenge with future potential then don't delay, ring me today and find out more. Alternatively, send or fax your CV to Dr Ian Collins, quoting ref. N/616/IC to:-

Executive Scientist Appointments Limited, 15–17 The Broadway, Old Hatfield, Herts AL9 5HZ. Tel: (0707) 264311. Fax: (0707) 275402.

(6127)A

E S A
EXECUTIVE SCIENTIST APPOINTMENTS

RECRUITING TOP PEOPLE IN SCIENCE

UNIVERSITY OF EDINBURGH
DIVISION OF BIOLOGICAL SCIENCES

1. Temporary Lectureship in Seed Technology

Applications are invited for a Lectureship in Seed Technology in the Institute of Ecology and Resource Management within the Division of Biological Sciences. The successful candidate will be expected to make a significant contribution to the MSc Course in Seed Technology and to undertake relevant research and consultancy work. The appointment is temporary, for one year in the first instance, but may be renewed on an annual rolling contract basis. The post is tenable from 1 October 1991. Salary will be within the range £12,086-£16,755.

Applicants should possess a degree in Agricultural Science, Crop or Plant Science, or Biology, have experience in Seed Technology and an interest in teaching at postgraduate level.

Informal enquiries can be made to Dr M R Turner, Director of the Seed Technology Course (Telephone 031-667 1041 ext. 4064).

2. Temporary Lectureship in Plant Biochemistry

Applications are invited for a Lectureship in Plant Biochemistry arising from the award of a Nuffield Research Fellowship to Dr S C Fry. The post will be held in the Institute of Cell and Molecular Biology within the Division of Biological Sciences. The successful candidate will be expected to teach undergraduates at 2nd, 3rd and 4th year level and will be encouraged to undertake research. The appointment is temporary, for one year only, and tenable from 1 October 1991. Salary will be within the range £12,086-£13,495.

Applicants should possess a degree in Plant Science, Biochemistry or Biology.

Informal enquiries can be made to Professor R. P. Ambler, Head of the Institute of Cell and Molecular Biology. (Telephone 031-650 5361).

Further particulars for these posts (quoting Ref. 1563 for Seed Technology and Ref. 1564 for Plant Biochemistry) can be obtained from the Personnel Office, University of Edinburgh, 1 Roxburgh Street, Edinburgh EH8 9TA, with whom applications including a full curriculum vitae (three copies) and giving the names of three referees, should be lodged by 24 May 1991.

(6149)A

UCLA Department of Physiology

Faculty Position

Regulatory and Integrative Physiology

We wish to appoint someone studying how cellular or molecular processes are governed by functional needs, such as those of the whole animal. We are not particularly concerned with what particular system the candidate studies. Instead, we seek someone with broad interests and perspective, demonstrated capacity to connect different levels of organization, and the ability to interact with physiologists studying various systems and organizational levels. The candidate may be either (1) a cell/molecular biologist accustomed to relating findings to higher level constraints, or (2) an organ, system or whole-animal biologist accustomed to relating findings to cell/molecular mechanisms. In addition, the appointee will be expected to particpate actively in the teaching mission of the department.

The search is without regard to level of appointment: established senior as well as junior candidates will be considered. Candidates should send a curriculum vitae, statement of interests, and 2-3 representative published papers, and the names of three referees, to:

Regulatory Search Committee
Department of Physiology
UCLA School of Medicine
Los Angeles, CA 90024-1751

The University of California is an Affirmative Action/Equal Opportunity Employer.

(NW6597)A

Postdoctoral Position University of Lausanne Department of Pharmacology

i) To study the molecular and cellular biology of the pancreatic β-cell glucose transporter (GLUT-2), ii) to determine by expression cloning the structure of β-cell-specific hormone/neuropeptide receptors and study their function.

Candidates should have a strong background in the analysis of proteins expression and/or in recombinant DNA technology. The position is available starting July 1st, 1991 and is funded for three years. Starting annual salary Sfr. 51,000.-

Send c.v. and the addresses of 2 references to Dr. Bernard Thorens, Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02140, USA. (NW6680)A

INSTRUCTOR IN PATHOLOGY

Instructor in Pathology to teach graduate students and fellows and perform related research in receptors on natural killer cells. Requires Ph.D. in Chemistry or Biochemistry with at least two years postdoctoral training in gene cloning, PCR, transfection, and site directed mutagenesis. Strong background in protein chemistry is highly desirable. Candidates currently holding junior faculty position preferred. **Send CV and 3 letters of reference to: Vinay Kumar, M.D., Department of Pathology, UT Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, Texas 75235-9072.** *An Equal Opportunity Employer.* (NW6682)A

POSTDOCTORAL POSITION

Position available for a biochemist/molecular biologist to participate in studies of plasminogen activator inhibitor-1, a protein which plays a critical role in the regulation of blood coagulation and a number of other important biologic processes. Projects include structure/function analysis of recombinant proteins and the use of transgenic and ES-cell technologies to develop specific animal models. Please send a description of research interests, curriculum vitae, and names and telephone numbers of three references to: **Dr. David Ginsberg, University of Michigan, 1150 West Medical Center Drive, 4520 MSRB I, Ann Arbor, MI 48109-0650.** *Equal Opportunity Employer.* (NW6678)A

Research Chemist

NMR Spectroscopy

Department of Insulin Research (Diabetes Division, Bagsvaerd) expands the use of NMR spectroscopy for research in structure-function relationships for insulin, insulin analogs as well as other peptides and receptors relevant for diabetes research.

The Department installs a 600 MHz instrument this fall and is searching for a Research Chemist (Ph.D. with relevant postdoctoral experience) to fill the position being technically responsible for the operation and maintenance of the facility.

The Candidate is further expected to participate in research projects groups and contribute substantial knowledge in the area of protein structure/physical chemistry. Experience with one or more thecniques commonly used in protein NMR is required, e.g., the sequential assignment approach, the use of heteronuclear multidimensional NMR, and the determination of threedimensional structures from NMR data. A working knowledge on molecular modelling is preferable.

The position involves extensive interactions with other members of the department, and support to NMR users from other divisions is also part of the job. Collaborative skills are therefore essential.

Candidates should send their application labeled „NMR spectroscopist 208" to

Novo Nordisk

Novo Nordisk A/S
Health Care Group, Personnel Department
Krogshoejvej 31
DK-2880 Bagsværd, Denmark

Novo Nordisk A/S is one of the world's leading biotechnology companies. It is a major force in insulin manufacture and diabetes treatment and is the world's largest producer of industrial enzymes. The company also manufactures and markets a variety of other pharmaceutical and bioindustrial products. Headquartered in Denmark, Novo Nordisk employs more than 8.000 people in 30 countries.

(W8439)A

Tokyo Medical & Dental University

A tenure junior faculty position. Focus is on molecular basis of hormone/growth factor actions in bone cell differentiation. **Send CV to: Prof. Masaki Noda, Tokyo Medical & Dental University, 3-10, Kanda - Surugadai 2-chome, Chiyoda - ku, Tokyo 101, Japan.** (W8441)A

INSTRUCTOR IN PATHOLOGY

PhD in basic sciences or MD with special significance in molecular genetics and post-doctoral fellowship and experience in recombinant DNA technology required. Duties to include teaching graduate students and fellows. **Send CV and 3 letters of reference to: Errol Friedberg, MD, Department of Pathology, UT Southwestern Medical Center at Dallas, Texas 75235-9072.** *Equal Opportunity Employer.* (NW6684)A

Department of Zoology
UNIVERSITY OF OXFORD
NERC Unit of Behavioural Ecology
Research Assistantship (1B) in theoretical and experimental behavioural ecology.

Applications are invited for the above post of graduate research assistant, to work with Dr A I Houston on mathematical modelling in behavioural ecology and/or laboratory experiments with birds. Some mathematical background would be an advantage. The successful applicant might have the opportunity to register for a higher degree. The start date is 1st October 1991 and the salary is on the RA1B scale (£11,399-£15,444 under review).

Applications, including a CV and names of 2 referees should be sent by 7th June 1991 to Mrs F Ryan, NERC Unit of Behavioural Ecology, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, from whom further details may be obtained.

Oxford University is an equal opportunities employer.

(6122)A

POSTDOCTORAL POSITIONS

available to study cell and molecular biology of insulin and growth factor action. Focus on receptor function, turnover and regulation. Canadian immigration requires priority be given to Canadian citizens and permanent residents of Canada.

Send CV and three reference names to: Dr Barry I Posner, Room M9.05, Royal Victoria Hospital, 687 Pine Ave. West, Montreal, Quebec H3A IA1.

(NW6691)A

The Research Centre for Arthritis and Autoimmunity announces positions for

Five Senior Staff Scientists

Located at The Wellesley Hospital and affiliated with The Department of Immunology, University of Toronto

The Centre has been formed to study the fundamental components of the immune system and thereby contribute to better understanding of Autoimmune Disease. We are currently seeking highly motivated individuals with research interests in:

- BIOCHEMISTRY: Assembly, processing, structure/function relationship of proteins such as the MHC, antigen receptors, signal transduction molecules, hormone receptors, growth factors/receptors.
- MOLECULAR BIOLOGY: Genetic mechanisms which initiate and regulate the immune response, repertoire formation, lymphocyte growth and differentiation, and cellular interactions.
- CELLULAR BIOLOGY: Cellular regulation of immunity, models of autoimmune disease, mechanisms of cell selection and expansion.

In the selection of candidates consideration will be given to: a demonstrated track record of producing high quality science; the ability to develop and maintain independent, externally funded research programs; a willingness to interact with other members of the Department, a citywide group of 40 scientists, and with the clinical research staff of The Wellesley Hospital in Endocrinology, Rheumatology, Inflammation Research; and evidence of teaching ability. A PhD or eqivalent degree, as well as several years postdoctoral experience, are required. Academic rank, from assistant to full professor, the the Department of Immunology will be determined based on qualification and experience. A competitive package including salary, benefits, space, and start-up funds will be provided starting July 1, 1991. Interested individuals should send, by July 1st, 1991, their curriculum vitae and a few representative publications to: **Dr Christopher J Paige, Director, The Wellesley Hospital Research Institute, 160 Wellesley Street East, Toronto, Ontario M4Y 1J3.**

The Wellesley Hospital is a fully affiliated University of Toronto Teaching Hospital.

(NW6690)A

Department of Experimental Medicine
COLLLEGE DE FRANCE – PARIS – FRANCE
POSTDOCTORAL RESEARCH POSITION

Available for 1 or 2 years to study the action of angiotensin II in transmembrane signaling. The approach involves combined application of recombinant DNA techniques and biochemical analysis of second messengers, including unicellular recording of Ca transients. Expertise in these fields will be an advantage.

French citizen applications cannot be considered.

Salary range: FF 100.000 (according to age and experience).

Please send CV + name of two references to:
Doctor Eric Clauser — INSERM U36 (Pr P. Corvol), Collège de France - 3, rue d'Ulm - 75005 Paris, France.

(W8448)A

TROPOSPHERIC STUDIES FACULTY POSITION

University of Michigan
Department of Atmospheric, Oceanic and Space Sciences

Tropospheric Studies. The University of Michigan Department of Atmospheric, Oceanic and Space Sciences invites applications for three tenure track faculty positions. Areas of specialization include, but are not limited to, climate dynamics, synoptic and mesoscale meteorology, atmospheric chemistry, radiative transfer, boundary-layer studies and dynamics. Candidates in both experimental and theoretical areas are sought. The successful candidate will be expected to teach undergraduate and graduate courses, develop a strong research program, and interact with the University's expanding Global Change Program.

The available position may be filled at any rank, commensurate with the qualifications of the applicant. Applicants should submit a complete resume, statement of future research and teaching interests, and names of at least three references to: **Perry J Samson, Department of Atmospheric, Oceanic and Space Sciences, 2126 Space Research Building, University of Michigan, Ann Arbor, Michigan 48109-2143.**

Applications received by June 15, 1991, will receive full consideration.

Phone: 313-763-6213. E-Mail: samson@madlab.spri.umich.edu.

The University of Michigan is an equal opportunity/affirmative action employer and educational institution. (NW6686)A

KAROLINSKA INSTITUTE
Department of Medical Nutrition
POSTDOCTORAL RESEARCH ASSISTANT
(THREE POSITIONS)

The Department of Medical Nutrition is a university department within the Karolinska Institute School of Medicine with a staff of some 100 persons (scientists, students, technical and administrative staff). The main research areas include: steroid hormone receptors (structure, regulation and gene expression), dioxin receptor (structure, function and gene expression), chemical hepatocarcinogenesis, molecular mechanism of growth hormone regulation, cytochrome P-450 in the central nervous system and environmental factors in the etiology of human cancer. The three postdoc positions relate to growth hormone mechanism of action, P-450 in the CNS and endocrine regulation of chemical hepatocarcinogenesis.

The position as postdoctoral research assistant is initially for two years with possibilities for extension up to four years. Preference is given to applicants with experience in molecular biology. Adequate laboratory resources will be provided.

Applications should be sent to Professor Jan-Åke Gustafsson, Dept. of Medical Nutrition, Karolinska Institute, Huddinge University Hospital, NOVUM, S-141 86 Huddinge, Sweden, together with CV, relevant publications and names of two referees.

(W8434)A

UNIVERSITY OF READING
School of Plant Sciences
PLANT TISSUE CULTURE

Applications are invited for a Research Fellow to carry out research related to plant tissue culture and to increase the School's potential for external funding.

The Fellow will develop contacts with commercial companies, run short courses and provide suitable training. The appointment is for a fixed period of three years in the first instance.

Candidates should have experience of working with *in vitro* systems and a Ph.D in a relevant area. Starting salary up to £14,038 p.a.

Apply for further particulars and application form (2 copies) to Personnel Officer, University of Reading, Whiteknights, P.O. Box 217, Reading RG6 2AH. Telephone (0734) 318754.

Closing date: 24 May 1991.

Please quote Ref: R9123. (6125A)

UNIVERSITY OF SHEFFIELD
Department of Biomedical Science
RESEARCH ASSISTANT (GRADE B)

Applications are invited for a three year post funded by the British Diabetic Association. The project will investigate the receptor-operated control of ion channels in pancreatic-cells using patch-clamp techniques.

Candidates should have or expect to receive a good honours degree in Physiology, Biochemistry or a related discipline.

The successful applicant will be expected to register for a higher degree. Salary will be on the IB scale for Research & Analogous Staff (from £11,399 pa).

Applications, including a CV and the names/addresses of two referees to: Dr M J Dunne, Department of Biomedical Science, University of Sheffield, Western Bank, Sheffield S10 2TN. Informal enquiries welcome. Please quote Ref: R1112/G.

A Equal Opportunity Employer

(6144)A

UNIVERSITY OF PENNSYLVANIA SCHOOL OF MEDICINE

The Department of Biochemistry and Biophysics plans to appoint a tenure track assistant professor with research interests in the area of molecular evolution, RNA catalysis and metabolic engineering.

Candidates should submit a curriculum vitae and statement of research interests, and have 3 letters of recommendation sent to: Dr. Franz Matschinsky, Department of Biochemistry and Biophysics, 414 Anatomy-Chemistry Building, University of Pennsylvania, Philadelphia, PA 19104-6059.

Deadline for applications is June 15, 1991.

The University is an Equal Opportunity/Affirmative Action Employer. (NW6681)A

COTTON-TOP TAMARINS PRIMATE DISEASE MODEL

The Center for the Study of Inflammatory Bowel Disease (CSIBD) at the MGH and the NERPRC maintains a large colony of Cotton-Top Tamarins (CTT) (*Saginus oedipus*) for use in research related to inflammatory bowel disease and colon cancer. Funded investigators interested in undertaking research projects involving non-terminal studies in the CTT are encouraged to contact the Primate Model Core Steering Committee, CSIBD, Massachusetts General Hospital, Jackson 7, Boston, MA 02114 or call (617) 726-7438. (NW6655)A

RESEARCH ASSOCIATE

PhD in Organic Chemistry. Classical carbohydrate manipulation; and organic synthetic methodologies relating to complex molecules. Must be familiar with the use of analytical procedures, such as proton and carbon NMR, UV, IR, GC/MS, LC, TLC, computer-assisted molecular modeling for structure determination of complex natural products. Publication record showing independent research required.

Salary: $22,350 @ 40 hours. If not a US citizen indicate visa status and basis for work authorization in the US.

Apply to: Employment Security Commission, 516 North Mangum Street Durham, NC 27702 or your local Job Service Office. Refer to DOT Code 090.227.010, Job Order Number NC 3001644 with copy of the advertisement within 30 days.

Employer: Duke University is an Affirmative Action/Equal Opportunity Employer (NW6669)A

UNIVERSITY OF THE WES INDIES

Cave Hill Campus, Barbados

Applications are invited for tl following posts in the Centre f Resource Management and Env ronmental Studies (CERMES):

FISHERIES AND RESOURCE MANAGEMENT

RESOURCE ECONOMIST

The successful applicants will b expected to assume duties as soon a possible. The posts are at the level Senior Lecturer/Lecturer. De pending on the qualifications an experience of the applicants one ap pointment will be made at the lev of Senior Lecturer and the other a the level of Lecturer. Proven re search ability and capacity to attai grant funding are essential, and th ability to teach data management, knowledge of microcomputer sy tems and Caribbean or other trop cal experience are highly desirabl Additional duties will includ supervision of students' project and organising and conductin training workshops in the Carib bean region. Salary Scales: Senic Lecturer BDS$54,840 × 1776 - 65,496 x 1920 – 71,256 (Bar) 2 1920 – 75,096 p.a. Lecture BDS$41,316 × 1776 – 51,97 (Bar) × 1776 – 62,628 per annum Up to five full economy class pas sages plus baggage allowance c US$1,200 on appointment and non mal termination. Special allowanc up to US$400 for shipment of aca demic books and teaching/researc equipment on appointment. Unfur nished accommodation at 10% c basic salary or optional housin allowance of 20% of basic salary t staff making own housing arrange ments. UWI contribution of equi valent of 10% of basic salary t Superannuation Scheme. Annua Study and Travel Grant for self spouse and up to three children Book Grant up to BDS$850 pe annum. Detailed applicat (three copies) giving full particular of qualifications and experience date of birth, marital status and th names and addresses of three re ferees should be sent as soon a possible to the Campus Registrar University of the West Indies, PO Box 64, Bridgetown, Barbados. Th University will send further particu lars for this post to all applicants These particulars may also be ob tained from the Appointments Of ficer, Association of Common wealth Universities, 36 Gordon Square, London WC1H 0PF, UK.

(W8418)A

UNIVERSITY OF ZIMBABWE

Applications are invited for the following post:

ASSOCIATE PROFESSORSHIP/SENIOR LECTURESHIP/ LECTURESHIP

DEPARTMENT OF SOIL SCIENCE AND AGRICULTURAL ENGINEERING

The successful candidate will be required to teach Pedology and Introductory Soil Science.

Salary per annum: Associate Professor Z$44,208-Z$46,500; Senior Lecturer Z$38,964-Z$42,096; Lecturer Z$28,080-Z$37,932. Both permanent and short-term contracts are offered. Persons who ar not Zimbabwean citizens may be appointed only on a short-term contract basis for an initial period of two years. Short-term contracts may be extended. Applicants should quote reference number ASA/8/4/91 and submit six copies of application giving full personal particulars which should include full name, place and date of birth, qualifications, employment and expeience, present salary, date of availability, telephone number and names and addresses of three referees to the Director, Appointments and Personnel, University of Zimbabwe, PO Box MP 167, Mount Pleasant, Harare, Zimbabwe. Telegrams: UNIVERSITY; Telex: 26580 UNIVZ ZW. Applicants from outside Zimbabwe should also send a copy to Appointments (39354), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF, UK, from whom further details may be obtained.

Closing date for receipt of applications is 24 May 1991. (W8437)A

UNIVERSITY OF NEWCASTLE UPON TYNE
Department of Biochemistry and Genetics

POSTDOCTORAL RESEARCH ASSOCIATE IN ENZYMOLOGY

Applications from biochemists or chemists are invited for a three-year appointment within a programme supported by the SERC. This post will be involved in the characterisation of the active site and catalytic mechanism of penicillin acylase to underpin protein engineering of the enzyme. The project will provide training in a range of techniques in protein-biochemistry with relevance to biotechnology.

Salary will be up to £13,495 per annum on the Grade 1A scale: (£11,399 — £18,165), according to qualifications and experience, and the appointment will commence as soon as possible.

Enquiries and applications, including a curriculum vitae and the names of three referees to Dr R Virden, Department of Biochemistry and Genetics, University of Newcastle upon Tyne, NE2 4HH (Tel: 091 222 7432 or 091 222 6000, extension 8126) to arrive not later than 31st May, 1991. (6157)A

DEPARTMENT OF GEOLOGY
University of Alberta

Applications are invited for a tenure track faculty position in Hydrogeology and/or Environmental Geology. The Department of Geology is a well-equipped research oriented department in the Faculty of Science. The successful candidate will be responsible for carrying on an independent research program, supervising graduate students and teaching undergraduates in the areas of hydrogeology environmental geology and introductory geology.

We are seeking a candidate who will be able to enhance an existing program in this area. Preference will be given to a candidate who has expertise in one or more of the following: groundwater flow and transport modelling, low temperature aqueous geochemistry, aquifer- and well-hydraulics, contaminant hydrogeology.

The position is at the assistant professor level ($36,910-53,374) and is available at the beginning of July 1992. Applications, including a curriculum vitae with education and work experience, the names and addresses of three referees, and a brief statement of teaching and research interests, should be received by July 31, 1991 and addressed to: **Dr. B.D.E. Chatterton, Chair, Department of Geology, University of Alberta, Edmonton, Alberta, Canada T6G 2E3.**

In accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada.

The University of Alberta is committed to the principle of equity in employment. The University encourages applications from aboriginal persons, members of visible minorities and women. (NW6665)A

Washington
WASHINGTON UNIVERSITY IN ST LOUIS

TENURE TRACK ASSISTANT PROFESSOR

MOLECULAR BIOLOGY AND PHARMACOLOGY

Washington University School of Medicine is recruiting an individual at the level of Assistant Professor in the tenure track. Outstanding scientists working within the broad area of molecular biology will be considered with an emphasis on those who have a strong background in mammalian genetics and who are expert at using transgenic mice to examine fundamental questions in gene regulation and function.

Interested applicants should forward a curriculum vitae, a short summary of research plans, and the names and addresses of three references to: Search Committee, c/o Jeffrey Gordon, Head, Department of Molecular Biology and Pharmacology, Box 8103, Washington University School of Medicine, 660 South Euclid Avenue, St Louis, MO 63110.

Washington University is an Affirmative Action/Equal Opportunity Employer. (NW6687)A

ars Agricultural Research Service — United States Department of Agriculture

The Agricultural Research Service (ARS), the principal research agency of the United States Department of Agriculture is accepting applications for

OVER 100 POSTDOCTORAL RESEARCH POSITIONS, NATIONWIDE

in genetics, molecular biology, chemistry, agronomy, and other disciplines.

There are some restrictions when hiring non-US citizens. Salary for the positions ($31,116-$37,294) is based on qualifications/ experience. Initial appointment up to 2 years.

Position descriptions and application materials may be obtained from N L Bakes, Personnel Division (NA), USDA-ARS, Room 139, 6305 Ivy Lane, Greenbelt, Maryland 20770. ARS is an equal opportunity employer. (NW6688)A

Advertisement for Faculty Positions in Molecular Regulation of Cellular Signalling

The Department of Pharmacology at the Duke University Medical Center is increasing its faculty as a part of a major expansion in the basic sciences. The department wishes to enhance its focus on molecular regulation of cellular signalling. Applicants are being sought who have a research background utilizing modern molecular biological and/or genetic technology and are interested in working in a collegial and interactive environment. Most appointments will be at the level of assistant professor. Please send curriculum vitae, list of three references and a brief description of proposed research to: **Dr. Anthony R. Means, Chairman, Department of Pharmacology, Box 3813, Duke University Medical Center, Durham NC 27710.** Duke University is an Equal Opportunity/Affirmative Action Employer. (NW6679)A

University Department of Neurology
Institute of Neurological Sciences,
Southern General Hospital, Glasgow.

RESEARCH ASSISTANT GRADE 1A

Applications are invited for the post of Research Assistant for a three year period funded by the Scottish Motor Neurone Disease Association. The purpose of the project is to characterize the immunoglobulin variable region gene repertoire encoding anti-ganglioside autoantibodies from patients with motor neuron diseases. Candidates should either possess an honours degree in the biological sciences or a PhD and preferably have practical experience in DNA amplification, cloning and sequencing.
The starting date is around June 1991.
Salary is on first point of Grade 1A salary scale.
Applications with the names and addresses of two referees should be sent to **Dr. H. J. Willison, Lecturer, University Department of Neurology, Institute of Neurological Sciences, Southern General Hospital, Glasgow (Tel: 041 445 2466)** to whom informal enquiries can be made.
Closing date: 19th May, 1991. (6161)A

DEPARTMENT OF CELLULAR BIOLOGY
University of Geneva
FACULTY OF SCIENCES
Applications are invited for

ONE/TWO PROFESSORSHIPS
(Full Professor, possibly Adjunct Professor)
in cell biology

These professorships will be available in the new Department of Cell Biology at the University of Geneva. This department is accommodated in a modern Biology building.
The successful candidates will be expected to participate in both undergraduate and graduate teaching, and to establish a strong independent research program.
Applications are particularly invited from candidates whose areas of research interests include: Control of proliferation and development, cellular interaction and communication, intracellular traffic, compartmental interactions, organelle biogenesis, cell motility and cyto-architecture. However, outstanding biologists whose research interests are outside of these areas will also be considered. The language of instruction is French, but a period of adjustment to this requirement can be granted. The proposed starting date is January 1992, but this is negotiable.
Applications should be sent by July 31, 1991 to: Secrétariat de la Faculté des Sciences, Université de Genève, 30, quai Ernest-Ansermet, CH-1211 Geneva 4, Switzerland, where inquiries also can also be obtained. (W8452)A

ASSISTANT RESEARCH SCIENTIST OF SURGERY/OTOLARYNGOLOGY
UNIVERSITY OF CALIFORNIA, SAN DIEGO.

The UCSD Cancer Center Head & Neck Oncology Program under the joint sponsorship of the Divisions of Otolaryngology/Head and Neck Surgery and Hematology/Oncology (UCSD Pharmacology program) is seeking a full time research scientist with demonstrated expertise in molecular biology to head a new basic science program in the UCSD Cancer Center. Qualifications: Ph.D. in basic sciences with expertise in biochemical, immunological, molecular biology, and cell culture techniques. Responsibilities: Lead basic science research program for head and neck cancer, interact with other research programs/clinicians, obtain extramural grant funding. Salary per published UC scale. Closing date 6/15/91. Send curriculum vitae, publications and names and addresses of five references to **K. Thomas Robbins, M.D., Director, Head and Neck Oncology, UCSD Cancer Center (H-891-B), 225 Dickinson Street, San Diego, CA 92103. (619) 543-2708.** *AA/EOE.* (NW6683)A

UNIVERSITY OF BRISTOL
H.H. Wills Physics Laboratory

POSTDOCTORAL RESEARCH ASSISTANT 1A

Applications are invited for this position in scanning tunnelling microscopy to study biological molecules with Dr M J Miles and Professor E D T Atkins. The research project will involve an investigation of image contrast, tunnelling spectra and the mechanism of molecular adsorption to the substrate. It is proposed to develop the instrument to incorporate atomic force and scanning ion-conductance microscopy. The appointment, which has been awarded by the AFRC, is for up to 2½ years with a starting salary of up to £14,038 per annum (salary scales under review from 1.4.91) depending on age and experience.
Informal enquiries can be made to Dr Miles on 0272 303030, ext. 3618.
For further details telephone Bristol 256450 (ansaphone after 5.00 pm) or write to the Personnel Office, Senate House, Bristol, BS8 1TH. Please quote reference 674.
An Equal Opportunities Employer. (6154)A

FELLOWSHIPS/STUDENTSHIPS

UNIVERSITY OF NEWCASTLE UPON TYNE
Faculty of Medicine

RESEARCH STUDENTSHIPS

Applications are invited for research studentships leading to the degree of PhD, from candidates who possess, or expect to graduate with, a first or upper second class degree in a related subject. The studentships are available from 1st October 1991 and are tenable for three years. Projects available are:

1. Altered synaptic proteins in human muscle disease. (Dr. C. Slater — Tel: 091 273 8811, ext. 22180).
2. A physiological role for P-glycoprotein in epithelial tissue. (Dr. N. Simmonds — Tel: 091 222 6000, ext. 6999).
3. An investigation of phenytoin metabolism and its relation to the development of gingival hyperplasia. (Dr. F. Kamali — Tel: 091 222 6000, ext. 8043).
4. Role of type 1 insulin-like growth factor receptor in breast cancer. (Dr. B. Westley — Tel: 091 222 6000, ext. 7200).

Applications, stating the project of interest, and including full Curriculum Vitae and names of two referees, should be sent to **Ms S. Knauer, Administrative Assistant, Medical School, Framlington Place, Newcastle upon Tyne, NE2 4HH, by Friday 24th May, 1991.** Informal enquiries will be welcomed by the named supervisor.
(6158)F

Postdoctoral Fellowship Program

We are pleased to announce the Postdoctoral Fellowship Program for the newly formed Du Pont Merck Pharmaceutical Company. Du Pont Merck is a partnership between its parent companies, E.I. du Pont de Nemours and Merck & Co., Inc. Postdoctoral research will play a key role in our success.

The strong background in research, drug discovery and drug development of the parent organizations are the building blocks for Du Pont Merck. We look forward to creating a research-based, independent pharmaceutical firm which will occupy a leading position in today's global pharmaceutical industry. To accomplish this, selected Postdoctoral candidates will conduct research in the areas of our scientific interest, publish in scientific journals, attend and present their work at scientific meetings.

Du Pont Merck is seeking motivated individuals for two-year research programs in the following areas:

- **Chemical Sciences**
- **Biotechnology**
- **Pharmacology**
- **Viral Diseases**
- **Cancer and Inflammatory Diseases**
- **Cardiovascular & Central Nervous System Diseases**
- **Drug Metabolism**
- **Toxicology & Safety Assessment**
- **Radiopharmaceuticals**
- **Rational Drug Design**
- **Biophysics**
- **Chemical Process Development**

The Fellowship Program offers a comprehensive package including a novel and exciting training environment, competitive salaries and medical benefits. For prompt, confidential consideration, forward a CV and statement of research interests and career goals to:

Dr. Lynn W. Enquist, Program Coordinator, The Du Pont Merck Pharmaceutical Company, Dept. NAT-16, P.O. Box 80328, Wilmington, DE 19880-0328. An equal opportunity employer M/F/H/V.

DU PONT MERCK

THE DU PONT MERCK PHARMACEUTICAL COMPANY
Merging Strengths...Emerging Opportunities

(NW6694)E

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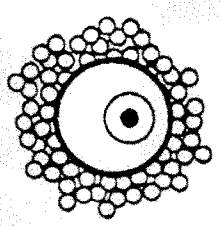

The Jackson Laboratory
announces

The Second Biennial Mammalian Developmental Genetics Workshop

11-15 September 1991

Keynote Addresses: The objective of these lectures will be to present the background and key questions in various areas of developmental genetics as well as to highlight the current research ongoing in the laboratories of the speakers.

A McMahon — Genes that control development in insects and mammals
S Waelsch — Mouse developmental mutations
D Solter — Imprinting
N Hecht — Genetic control of gametogenesis
B Hogan — Genetic control of morphogenesis
M Buckingham — Genetic control of lineage determination
A Joyner — Functional analysis of genes through gene targeting
V Papiaoannou — genetic control of implantation — are there possibilities?
J Hall — Human developmental genetics

Follow-up Speakers: After the keynote speaker in each session, there will be three 20 minute presentations emphasizing more detailed aspects of each subject. We invite the submission of abstracts (one page) in the areas of the Keynote Addresses from which these follow-up speakers will be selected. Speakers selected for the follow-up papers will receive $500 to help defray travel expenses and the registration fee will be waived.

Students: Limited funds are available to help Pre- and Postdoctoral students with travel expenses. Those interested should send a one page abstract of their research project and have their advisor send a letter of recommendation.

Schedule: There will be a reception on the evening of 11 September and sessions will begin on the morning of 12 September. There will be facilities for poster presentations (ask for more details). The meeting will end with a lobster banquet on the evening of 15 September.

Registration Fee: $400 (US) includes Reception, 3 Lunches, and Banquet.

Deadline for Abstracts and Applications: 15 July 1991.

Organizers: Joe Nadeau and John Eppig.

Information: *Those interested in participating should contact Phyllis Mobraaten at The Jackson Laboratory, Bar Harbor, ME 04609 USA, phone (207) 288-3371 or Fax (207) 288-5079 for further information.*

(NW6685)V

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UNIVERSITY COLLEGE LONDON

Department of Biochemistry & Molecular Biology

POSTGRADUATE STUDENTSHIPS

Studentships have been awarded to the Department of Biochemistry and Molecular Biology from MRC, AFRC and SERC. Applications will be welcomed for the following topics:

1) Nutritional biochemistry of tryptophan, niacin and vitamin B6; amino acid metabolism in schistosomiasis (bilharzia)
2) Mathematical analysis of biological systems
3) The properties of a mitochondrial pore of relevance to reperfusion injury
4) Metabolic systems protecting against damage by free radicals
5) Ischaemic disease and the role of the newly discovered nucleotide storage compound, oligophosphoglyceroyl-ATP (OPG-ATP)
6) Processes involved in the regulation of fatty acid oxidation by cardiac muscle
7) The molecular basis of pattern formation in embryonic development
8) Regulation of c-*fgr* proto-oncogene expression and function of its product
9) Investigations of protein/nucleic acid interactions in the regulation of *Pseudomonas aeruginosa* gene expression
10) Novel homeobox/POU family transcription factors expressed in sensory neurons
11) Transcriptional and post-transcriptional regulation of viral and cellular gene expression
12) Yeast promoters induced at high cell density
13) Plasma membrane proteins induced by stress and the plasma membrane in cyclic AMP control and stress protection
14) Control of eukaryotic gene expression — drug and carcinogen metabolising proteins
15) Structure, function and molecular biology of glutathione transferases with emphasis on their role in preventing cancer and drug resistance in malignancy
16) Mechanism of action of carcinogenic nitrosamines and their role as a cause of human cancer
17) The enzymology of extremely thermophilic bacteria and the application of thermostable enzymes to bioconversions in non-aqueous solvents
18) Investigation of protein function at the molecular level
19) The dependence of function of proteins and enzymes on their quaternary structures
20) Biomolecular structure and modelling
21) The molecular biology of *Streptomyces* plasmids, extracellular enzymes and degradative genes from soil organisms.

Candidates, who must be from UK/EEC, should have or expect to obtain a 2.1 or 1st class honours degree and should apply as soon as possible. Application forms can be obtained from the Postgraduate Admissions Tutor, Department of Biochemistry & Molecular Biology, University College London, Gower Street, London WC1E 6BT, or telephone 071-380 7033 for further details.

(6170)F

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AWARDS

PRE AND POST DOCTORAL TRAINEESHIPS

A number of Pre and Post Doctoral Traineeships are available in the six departments of the College of Biological Science. Support for these awards is derived from i) A University-Industry Co-operative Training Program sponsored by The Natural Sciences and Engineering Research Council (NSERC) (Available to Canadians and Landed Immigrants); ii) International Fellowships (available only to Foreign Nationals) supported by NSERC; iii) Traineeships provided by Faculty Research grants. The research emphases of each department are as follows.

BOTANY

Physiology & plant molecular biology, ecology, and plant structure & development.

MICROBIOLOGY

Molecular aspects of life cycles, physiology, pathogenesis, ecology and biotechnological applications of viruses and prokaryotic and eukaryotic microorganisms.

MOLECULAR BIOLOGY & GENETICS

Gene expression, genetic recombination, ribosome structure/function, chromatin, developmental genetics, cytogenetics and biotechnology of plant and animal systems. Plant cell biology.

NUTRITIONAL SCIENCES

Experimental nutrition at all levels of biological organization, ranging from molecular biology to ecology. Current research interests include the role of nutrients in biological membranes, in detoxification processes, in the control of inter-organ nutrient partitioning and in regulation of immune function.

SCHOOL OF HUMAN BIOLOGY

Questions concerning i) mechanisms of skeletal muscle fatigue and metabolic regulation, ii) the neurophysiology and biomechanics of human movement and iii) human population biology are investigated using approaches ranging from cultured cells to field study of human populations.

ZOOLOGY

Population and community ecology, evolution and population genetics, environmental physiology and parasitology with a particular emphasis on aquatic organisms.

The University of Guelph is committed to an Employment Equity Program that includes special measures to achieve diversity among its faculty and staff. We therefore particularly encourage applications from qualified aboriginal Canadians, persons with disabilities, members of visible minorities and women.

Applicants holding a 1st class (A), or upper second class (B+) degree, wishing to pursue a post graduate degree, are encouraged to apply. Individuals with a Ph.D. and interest in any one of the above areas of research will be considered for post doctoral traineeships. Candidates should send a complete curriculum vitae, including research publications and the names of 3 referees to:

Dr. Bruce H. Sells,
Dean, College of Biological Science
University of Guelph
Guelph, Ontario, Canada N1G 2W1

(NW6689)N

Applications will be considered until all the positions are filled.

UNIVERSITY of GUELPH

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CONGRESS

The Vitamin Society of Japan

The First International Congress on Vitamins and Biofactors in Life Science

September 16-20, 1991, Kobe Japan

Plenary Lectures by A Meister, H F DeLuca, K Okuda, T C Stadtman, K Decker, L J Reed, H Sies, E E Snell, F M Huennekens, M Mino, and Y Nishizuka.

21 Symposia on vitamins B_{12}, D, E, K, B_6, C, A, biopterin, thiamine, eicosapentaenoic acid, G-CSF, biogenic amines, zinc, free radicals, oxidation-reduction, biotechnology etc.

Free Communications by Oral and Poster Presentation
Deadline for abstract, June 15, 1991.

Congress Secretariat
Professor Nobuhiko Katunuma
Institute for Enzyme Research,
The University of Tokushima
3-18-15 Kuramoto-cho, Tokushima 770,
Japan
Phone 0886-31-3111 extension 2551
Fax 0886-33-0771.

(W8444)Q

FELLOWSHIPS/STUDENTSHIPS continued

YAMANOUCHI RESEARCH STUDENTSHIP

A PhD studentship will be available in the Department of Medical Oncology, University of Manchester, from October 1991. The successful candidate will be joining a team interested in the role of the extracellular matrix in neovascularisation and will be using a variety of techniques in biochemistry, cell and molecular biology. Salary will be £8,400 in the first year, with the appropriate increments for the second and third years.

Applications, including a full CV and names and addresses of three referees, should be sent to Dr A M Schor, CRC Department of Medical Oncology, University of Manchester, Christie Hospital and Holt Radium Institute, Wilmslow Road, Manchester, M20 9BX, UK, from whom further information is available (tel. 061 445 8123, ext. 432). Closing date: 21st June, 1991. (6159)F

PhD Studentships in Plant Molecular Biology and Plant-Microbe Interactions

Wye College (University of London)

Applications are invited from students who hold, or will graduate this year with, a first or upper second class degree in biology, biochemistry or a related subject for three studentships in the Department of Biochemistry and Biological Sciences to commence in October 1991.

1. Cloning of genes involved in starch biosynthesis in the wheat grain and analysis of function in transgenic plants expressing antisense RNA. (SERC-CASE project with Dr Charles Ainsworth at Wye College and Dr Mike Burrell at Advanced Technologies (Cambridge) Ltd).
2. Molecular analysis of a gene involved in host-specific nodulation in *Bradyrhizobium*. (AFRC studentship with Dr Jim Beynon).
3. Cell signalling and responses to fungal penetration in onion epidermal cells. (AFRC studentship with Dr John Mansfield and Dr John Rossiter).

Informal enquiries can be obtained from the staff members involved at: Department of Biological Sciences, Wye College, Ashford, Kent TN25 5AH. Telephone: 0233 812401. For further particulars and application forms apply to the Registrar at the College. (6143)F

POSTDOCTORAL FELLOWSHIPS

in gastroenterological research available for two or more years beginning 1991. Faculty employs multidisciplinary approach using biochemical, cell/molecular biological, immunological, physiological and electrophysiological techniques. Applicant may focus on clinical and/or basic studies. Applicants should have MD or PhD. Send curriculum vitae and names of three references to N F LaRusso, MD, Program Director, NIH Training Grant, Mayo Clinic, Rochester, MN 55905. Mayo Foundation is an affirmative action and equal opportunity educator and employer. (NW6673)E

POSTDOCTORAL FELLOWSHIP

Available July 1991 to study the molecular and cellular biology of insulin action. Specific projects include structure/function relationship for the insulin receptor, the cellular trafficking of glucose transporter (GLUT 4) enriched vesicles, and the regulation and expression of genes conferring insulin responsiveness on the target cells of muscle and fat.

Please send a curriculum vitae and the names of 3 references to: **Dr Paul F Pilch, Department of Biochemistry, Boston University School of Medicine, 80 E Concord St, Boston, MA 02118.** (NW6649)E

UNIVERSITY OF EDINBURGH In Conjunction with the University of Oxford POST-DOCTORAL FELLOWSHIP IN CELL AND MOLECULAR BIOLOGY

A three year post-doctoral position is available, jointly held between Professor A J Trewavas and Dr M Fricker, to develop a procedure for fluorescence ratio imaging of calmodulin and to analyse the relocation of calmodulin during guard cell closure. The research will mainly be carried out in Edinburgh, starting date January 1st 1992. Salary is on the 1A scale and depends upon age and experience. The successful applicant would join a small, enthusiastic but highly experienced calcium measurement and imaging group and would have the opportunity to create a breakthrough in protein imaging technology.

Applications (CV + two referees) can be made to Professor A. J. Trewavas, Institute of Cell and Molecular Biology, Daniel Rutherford Building, The King's Buildings, Mayfield Road, Edinburgh EH9 3JH (Tel 031-650 5328), or Dr Mark Fricker, Plant Sciences, University of Oxford, South Parks Road, Oxford (Tel 0865 275 015) from whom further information can be obtained.

Please quote reference 5866.

(6133)E

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AWARDS continued

AWARDS FOR COLLABORATION IN EUROPE FOR ACADEMIC RESEARCH GROUPS 1992-1994

Applications are invited for awards by the CIBA-GEIGY Fellowship Trust beginning in the summer of 1992.

CIBA-GEIGY 'ACE' AWARDS

CIBA-GEIGY 'ACE' Awards will be made to research groups at United Kingdom or Republic of Ireland universities, polytechnics or comparable teaching institutions to stimulate collaboration with research groups at Continental European institutions in the field of chemistry, biochemistry, chemical technology, chemical engineering and biology. The awards will be made to assist the cost of short visits in each direction between the collaborating institutions by members of either of the two research groups involved.

It is the intention to make at least six awards for a period of two years each.

Value of the awards: £1,500 per annum.

Further details and application forms are obtainable from: The Secretary, The CIBA-GEIGY Fellowship Trust, Hulley Road, Macclesfield, Cheshire SK10 2NX. (Telephone: 0625 421933).

The closing date for applications is 1 November 1991. (6152)N

CIBA–GEIGY

FELLOWSHIPS/STUDENTSHIPS continued

UNIVERSITY COLLEGE LONDON
Medical Molecular Biology Unit and Institute of Cancer Research, Chester Beatty Laboratories

RESEARCH STUDENTSHIP

A three year PhD studentship is available in the MRC Aids Directed Programme to work with Dr. D. S. Latchman (UCL) and Dr. M. K. L. Collins (ICR) on a collaborative proposal studying the interaction of cellular transcription factors with the HIV regulatory region during T cell activation. The work will use a combination of molecular biology and cell culture techniques and will not involve the use of live virus. Applicants should have or expect to obtain at least an upper second class degree in an appropriate subject.

Applications (with CV and names of two referees) should be sent to **Dr. D. S. Latchman, Medical Molecular Biology Unit, Department of Biochemistry & Molecular Biology, Windeyer Building, Cleveland Street, London W1P 6DB.** (6142)F

PROPOSALS

The Procter & Gamble Company
University Animal Alternatives Research Program

A Call for Research Proposals

The Procter & Gamble Company is committed to the development and use of new methods for testing the efficacy and safety of drugs and consumer products that eliminate or reduce the use of animals or distress imposed on animals. The University Animal Alternatives Research Program will provide funds for research to develop such methods.

Funding: Up to $50,000 annually for up to 3 years. Three such grants will be awarded in **1992.**

Deadline for Application: September 1, 1991

Announcement of Recipients: January 1, 1992

Proposals will be accepted from any academic or non-profit medical research institution. The Company is especially interested in proposals in the following areas:

Efficacy Testing

- Analgesics
- Inflammation
- Diseases of the Oral Cavity
- Gastrointestinal Disorders
- Cardiovascular Disorders
- Bone Disorders
- Skin Disorders
- Respiratory Diseases
- Nutritional Disorders
- Computer Modeling

Safety Testing

- Ocular Irritation
- Acute Oral Toxicity
- Skin Irritation and Contact Sensitization
- Developmental Toxicity
- Respiratory Toxicity
- Neurotoxicity
- Computer Modeling

Enquiries and requests for application details should be directed to:

Program Administrator
University Animal Alternatives Research Program
The Procter & Gamble Company
Miami Valley Laboratories
P.O. Box 398707
Cincinnati, Ohio 45239-8707
Fax (513) 245-1153

(NW6692)X

FELLOWSHIPS/STUDENTSHIPS continued

UNIVERSITY OF GLASGOW
Wellcome Unit of Molecular Parasitology

MOLECULAR GENETICS OF GROWTH

Two postdoctoral fellowships are available to undertake a research project on the role of ODCase in the growth of chickens and mice. The project is funded for three years under an A.F.R.C. Linked Research Group between the Wellcome Unit and the A.F.R.C. Institute of Animal Physiology and Genetics (Edinburgh); one of the two positions would be located in Edinburgh.

The project will involve the use of a combination of gene isolation, gene sequencing, transfection, *in vitro* mutagenesis and transgenic techniques in order to study the regulation of expression of the ODCase gene and its role in determining growth rate and lean body mass. Previous work has established that increased levels of ODCase are correlated with selection for high growth rate and body mass in both chickens and mice; in addition, in mice, this has been shown to be associated with sequence alterations at the 5′ end of the structural gene.

Applications should be submitted to Professor A. Tait, Wellcome Unit of Molecular Parasitology, Bearsden Road, Glasgow, G61 1QH from whom further information can be obtained. Experience of molecular or biochemical techniques would be an advantage. Salaries will be on the 1A scale within the range £14,744 — £16,026 and the starting date is negotiable. (6162)E

FELLOWSHIPS/STUDENTSHIPS continued

CONTINENTAL EUROPEAN FELLOWSHIP AWARDS 1992-1993 FOR ACADEMIC STAFF

Applications are invited for awards by the CIBA-GEIGY Fellowship Trust for the academic year 1992-1993.

Senior CIBA-GEIGY Fellowships

Senior CIBA-GEIGY Fellowships will be awarded to start from an agreed date during the academic year 1992-1993. These will be available to lecturers, senior lecturers or readers who hold, and will return to, permanent teaching positions at United Kingdom or Republic of Ireland universities, polytechnics or comparable teaching institutions, who wish to undertake research at an agreed continental European university or technological university. These posts should have been held for a period of at least five years in the field of chemistry, biochemistry, chemical technology, chemical engineering and biology, particularly in its relation to chemistry.

Awards are made for a period of four to twelve months.

Value of the awards: £12,000 per annum plus travelling expenses.

CIBA-GEIGY Fellowship awards are intended to supplement Fellows' salaries for the period of tenure.

Further details and application forms are obtainable from: The Secretary, The CIBA-GEIGY Fellowship Trust, Hulley Road, Macclesfield, Cheshire SK10 2NX. (Telephone: 0625 421933).

The closing date for applications is 1 November 1991.

CIBA–GEIGY

(6153)E

INSTITUTE OF PSYCHIATRY
(University of London)
J. PAUL GETTY Jnr. Fellowship in the Psychiatry of Addiction Behaviour

The Institute of Psychiatry is establishing this Fellowship at Senior Lecturer level within the Addiction Research Unit (Director, Professor Griffith Edwards). Candidates must hold MRCPsych. or equivalent and will be eligible for an Honorary Consultant appointment at the Bethlem Royal and the Maudsley Hospitals. A significant record of research in this field is expected. Salary will be in the Clinical Senior Lecturer range £33,720-£43,100 pa.

For further written details contact the Personnel Office, Institute of Psychiatry, De Crespigny Park, London SE1 8AF telephone 071-703 5411 ext. 3164 (24 hours). Applications in the form of a CV including the names of two referees should be received by the Personnel Office by 24 May 1991. Please quote reference 91/A15.

Working Towards Equal Opportunities. (6137)E

AFRC INSTITUTE OF ANIMAL PHYSIOLOGY AND GENETICS RESEARCH
EDINBURGH RESEARCH STATION
RESEARCH STUDENTSHIPS EDINBURGH

Applications are invited now for a research studentship tenable for 3 years from 1st October 1991. The successful applicant will join the Bone Biology Programme and will work on a project investigating the role of Bone Morpohogenic Proteins and related growth factors in the regulation of osteoblast differentiation and function. Techniques will include in situ hybridization and tissue culture.

Applicants should have (or expect to obtain) a 1st or 2.1 honours deree in Biochemistry, Molecular or Cellular Biology or a related subject. The standard conditions of the AFRC will apply, including the newly enhanced stipend.

Applications including a full CV naming two referees should be sent as soon as possible to Dr Houston or Dr Thorp, AFRC Institute of Animal Physiology and Genetics Research, Roslin Midlothian RH25 9PS.

Informal enquiries tel 031 440 2726. (6131)F

AFRC INSTITUTE OF ANIMAL PHYSIOLOGY AND GENETICS
EDINBURGH RESEARCH STATION
AFRC/CASE PH.D. STUDENTSHIP: 1991

Applications are invited from students who have, or expect to obtain, a first or second class degree in Molecular Biology or a related discipline for a project to study the effects of chromosomal position on gene expression. The aim of the research is to determine whether flanking chromosomal sequences can be utilised to enhance the efficiency of gene expression. The project will involve cloning, gene transfer and the analysis of gene expression and will be under the supervision of Dr. J. Clark. As a collaborative award with Pharmaceutical Proteins Ltd the student will spend up to 6 months in the Company's Edinburgh Laboratories. The person appointed will be registered for a PhD with Edinburgh University and will be associated with the newly established Centre for Genome Research. The position is available from October 1991.

Applications (including a CV and the names of two academic referees) and any enquiries should be made to Dr. J. Clark, AFRC Institute of Animal Physiology and Genetics, Roslin, Midlothian EH25 9PS. Tel: 031-440 2726. (6124)F

EMBO
EUROPEAN MOLECULAR BIOLOGY ORGANIZATION
SHORT TERM FELLOWSHIPS
in molecular biology

The European Molecular Biology Organization awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration. The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance. Applications may be made at any time and are decided upon soon after the receipt of application.

Applications for exchanges between laboratories within any one country cannot be considered.

Inquiries should be accompanied by a self-addressed adhesive label.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022. 40, F.R.G. (W8054)E

UNITED MEDICAL AND DENTAL SCHOOLS OF GUY'S AND ST THOMAS'S HOSPITALS
(University of London)
DIVISION OF ANATOMY AND CELL BIOLOGY, GUY'S CAMPUS
RESEARCH STUDENTSHIP

A Research Studentship, funded by the Anatomical Society of Great Britain and Ireland, is available to investigate the cellular and molecular mechanisms of the interactions between regenerating axons and astrocytes in a tissue culture model system. The successful applicant will join an active research group working on various aspects of axon growth and regeneration. Applicants should have a good Honours degree in a Biological Science.

Informal enquiries may be made to Dr J Cohen (071-955 4420) or to Professor M Berry (071-955 4360). Applications, including a CV and the names and addresses of 2 referees, should be sent to Dr J. Cohen, Department of Anatomy and Cell Biology, UMDS Guy's Campus, London Bridge, London SE1 9RT by 31 May 1991. (6139)F

STA FELLOWSHIPS

Postdoctoral Fellowships in Japanese Government Laboratories

In 1988, the Japanese Science and Technology Agency (STA) established STA Fellowships Program to offer postdoctoral fellowships to scientists and engineers of the countries listed below, for periods of 6 months to 2 years to be held in any Japanese national laboratory (excluding university and university-affiliated laboratories). Management of the STA Fellowship, including recruitment of candidates, is currently entrusted to the Research Development Corporation of Japan (JRDC), a statutory organisation under the supervision of the STA. Over one hundred Japanese research laboratories covering almost all areas of science, engineering and medicine are participating in the scheme.

Research Fields

Mathematics; Physics; Geology; Electricity; Electrons; Information Engineering; Mechanics; Engineering Works; Architecture; Erosion Control; Landscaping; Chemistry; Metals; Resources Engineering; Medical Science; Pharmaceuticals; Agricultural Technology; Farming; Forestry; Fisheries; others in the Natural Sciences.

The fellowships are open to young PhD holders of under 35 in principle, from universities, research councils, government research laboratories and industry. Any science or engineering discipline will be considered except military R & D. Applicants will be required to supply a letter of acceptance from their Japanese host institutes (the organizations listed in Table 1 provide help in contacting suitable host institutes).

Within the JRDC there are no closing dates but candidates are encouraged to submit their applications as soon as possible.

FELLOWSHIP AWARDS

Fellowships include round-trip air tickets (economy class; not available for dependents) and the following tax-free allowances:

1) Living allowance: ¥ 270,000 a month
2) Family allowance: ¥ 50,000 a month
3) Housing: Apartments will normally be provided to awardees. The apartment floor are is 40m² for awardees unaccompanied by their family and 60m² for awardees to stay with their family in other than metropolitan areas such as Tokyo. If an awardee prefers to use a larger apartment because of the family size or otherwise ¥ 100,000 maximum per month may be paid as housing allowance; any shortfall is to be borne by the awardee.
4) An initial international settling-in allowance: ¥ 200,000
5) An annual allowance of up to ¥ 115,000 for travel within Japan related to research activities.

APPLICATION PROCEDURE FOR STA FELLOWSHIP

Responsible organizations overseas which represent the governments of their respective countries are given in Table 1.

A researcher wishing to be awarded the STA Fellowship should apply to the responsible organization in his/her country (only those with Permanent Resident Status in the countries listed below need apply). Candidates are required to contact the desired host institute and obtain a letter of acceptance before filling an application with their government. Further information regarding the STA Fellowship and host Institutes is available from the responsible organizations.

TABLE 1 RESPONSIBLE ORGANIZATIONS

AUSTRALIA	**DEPARTMENT OF INDUSTRY, TECHNOLOGY, AND COMMERCE** Ms. Linda Tindall-Mather, Senior Project Officer 51 Allara Street, Canberra ACT 2601 Tel: 61-62-76-2037 Fax: 61-62-76-1706
AUSTRIA	**FEDERAL MINISTRY FOR SCIENCE AND RESEARCH** Dr. Othmar Huber Head Freyung 1, 1014 Vienna Tel: 43-222-531-20 Fax: 43-1-53120-4499
CANADA	**NATURAL SCIENCE AND ENGINEERING RESEARCH COUNCIL** Ms. Dawn Conway Director, Int'l Programs 200 Kent Street, Ottawa, K1A 1H5 Tel: 1-613-995-6424 Fax: 1-613-992-5337
GERMANY	**ALEXANDER VON HUMBOLDT-STIFTUNG** Dr. Michael Meier Jean-Paul-Str. 12, D-5300 Bonn 2 Tel: 49-228-833-127 Fax: 49-228-833-199
FINLAND	**THE ACADEMY OF FINLAND** Dr. Elisabeth Helander Research Director P.O. Box 57, SF-00551 Helsinki Tel: 358-0-7758-230 Fax: 358-0-7758-299
FRANCE	**CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE** Ms. Françoise Aubujeault Chief of Asia Division in the Dept. of Int'l and Cooperation Relations 15 Quai Anatole-France, 75700 Paris Tel: 33-47-53-15-15 Fax: 33-47-53-00-55
ITALY	**MINISTERO DELLA RICERCA SCIENTIFICA E TECHNOLOGICA** **Ufficio Relazioni Internazionali** Dr. Maria Enrica Danubio Head of Scholarship Section of the Int'l Relation Office Lungotevere Thaon di Revel 76, 00100 Roma Tel: 39-6-396-5180 Fax: 39-6-392-209
NETHERLANDS	**STICHING VOOR DE TECHNISCHE WETENSCHAPPEN** Dr. C.A.M. Mombers Program Officer Postbus 3021, 3502 GA Utrecht Van Vollenhovenlaan 661, Utrecht Tel: 31-30-923211 Fax: 31-30-946099
NEW ZEALAND	**INTERNATIONAL SCIENCE UNIT** Ms. Adrienne Stanton Advisory Officer Dept. of Scientific & Industrial Research P.O. Box 1578, Wellington Tel: 64-4-729-979 Fax: 64-4-724-025
NORWAY	**THE ROYAL NORWEGIAN COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH (NTNF)** Ms. Else Boon Executive Officer P.O. Box 70, Tåsen 0801 Oslo 8 Tel: 47-2-237685 Fax: 47-2-181139 or 47-2-184137
SWEDEN	**STYRELSEN FOR TEKNISK UTVECKLING** Ms. Margareta Tiger Senior Administrative Officer (Planning Department), Box 43200 S-10072, Stockholm Office: Liljeholmsvägen 32 Tel: 46-8-775-4000 Fax: 46-8-19-68-26
SWITZERLAND	**SWISS NATIONAL SCIENCE FOUNDATION** Mr. Benno G. Frey Head of the Fellowship Section Wildhainweg 20, CH-3012, Bern Tel: 41-31-27-22-22 Fax: 41-31-23-30-09
UNITED KINGDOM	**THE ROYAL SOCIETY** Ms. Jackie Gordon Head of Japan Exchanges 6 Carlton House Terrace, London SW1Y 5AG Tel: 44-71-839-5561 Fax: 44-71-930-2170
UNITED STATES	**NATIONAL SCIENCE FOUNDATION** Dr. Larry H. Weber Program Manager, Japan Programs Division of International Programs 1800 G Street, NW., Washington D.C. 20550 Tel: 1-202-357-9558 Fax: 1-202-357-5839
EUROPEAN COMMUNITIES	**COMMISSION OF THE EUROPEAN COMMUNITIES** Mr. Mario Merla Director-General for Science, Research and Development, Joint Research Centre, DG XII-G-3, 200 Rue de la Loi, 1049 Brussels, Belgium Tel: 32-23-53990 Fax: 32-23-63308.

Research Development Corporation of Japan, (JRDC)
Address: 5-2, Nagata-cho 2-chome, Chiyoda-ku, Tokyo 100, Japan. (W8443)E

FELLOWSHIPS/STUDENTSHIPS continued

Dundee Institute of Technology

Department of Molecular & Life Sciences

AFRC Research Studentship

Sperm–egg Interaction In Birds

Applicants for the above studentship should hold, or expect to gain in the near future, a first or upper second class honours degree or equivalent attainment in a biological science.

The basic stipend, in the first year, will be £5,000 for a student living outwith the parental home.

The project, under the supervision of Dr G Wishart, will utilise a range of cytochemical and protein separation techniques. Opportunities will also exist for investigating the potential for manipulating fertilization with a view to enabling cryoconservation and transgenesis.

Application forms and further particulars may be obtained from the Personnel Officer, Dundee Institute of Technology, Bell Street, Dundee, DD1 1HG with whom completed application forms should be lodged by 15 May 1991.

(6115)F

UNIVERSITY OF OXFORD
MAGDALEN COLLEGE

The College proposes to elect a Visiting Fellow of Fellows for the academic year 1992/93. In addition, a Robert S. Campbell Visiting Fellow may be elected for research in law, especially commercial or competition law.

A Visiting Fellowship is intended to offer an established scholar, either from abroad or from the United Kingdom, an opportunity to pursue his or her own study and research as a member of the College.

Further details and application forms may be obtained from the Clerk to the College. Completed forms must be returned to the Clerk to the College, Magdalen College, Oxford, OX1 4AU not later than 25 January 1991.

(6138)E

UNIVERSITY OF OXFORD
Department of Zoology
RESEARCH STUDENTSHIPS AVAILABLE

NERC:
Behavioural Ecology
Write to Professor J R Krebs FRS Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS.

MRC:
Epidemiology, Cell and Development. *Write to Director of Graduates, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS.*

(6114)F

PRIZES

$5,000 PRIZE

A former NASA physicist is offering an award of $5,000 to the first person who can demonstrate that his concept of a space drive will not work. A strong physics background is required. **Please contact Prof James H Vance, 4 Rocky Hill Road, Amherst, NH 03031.**

(NW6651)U

WORKSHOPS continued

EMBO-FEMS-NATO WORKSHOP
BACTERIAL PLASMID-CODED TOXINS
BACTERIOCINS, MICROCINS AND ANTIBIOTICS

The Island of Bendor, France. September 22nd-26th, 1991
Organizers: Dr Richard James, *Norwich, UK*
Prof. Claude Lazdunski, *Marseille, France*
Dr Franc Pattus, *Heidelberg, Germany*

Speakers include:
V Braun (Tubingen, W A Cramer (Purdue), A Finkelstein (New York), R E Glass (Nottingham), S P Howard (Newfoundland), T Itoh (Osaka), K Jakes (New York), R James (Norwich), R Kadner (Charlottesville), T R Klaenhammer (N Carolina), R Kolter (Boston), L Letellier (Paris), P Lau (Montreal), C Lazdunski (Marseille), J Luirink (Amsterdam), H Masaki (Tokyo), F Moreno (Madrid), B Oudega (Amsterdam), F Pattus (Heidelberg), R E Webster (N Carolina).

Applications
Applications should be sent to **Dr Richard James, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, Norfolk, UK (Fax 0603 259492) before June 30th, 1991.** Please include a short curriculum vitae and specify your scientific experience and interest in the theme of the workshop. A registration fee of 2500FF will be requested to cover registration and board and lodging. Applicants under 35 who are members of societies affiliated to FEMS may receive assistance with travel expeneses.

(6121)V

COURSES

AN INTRODUCTION TO *IN SITU* HYBRIDISATION

Organised by Gary Coulton

To be held at: Department of Biochemistry, Charing Cross & Westminster Medical School, Fulham Palace Road, London W6 8RF.

10-11th July 1991.

This course will introduce the fundamentals of *in situ* hybridisation. The course is based on a series of lectures and practical demonstrations and will be valuable for anyone in medical, biological or forensic fields.

Further details contact, Mrs Shirley Line at the above address or phone 081-846 7048 or Fax 081-846 7099: Fee £100 or £125 to profit-making organisations.

(6147)D

Techniques in Plant Molecular Biology

September 9th-18th 1991

Plant Molecular Biology Laboratory
Wye College (University of London)

This ten day intensive practical course is designed to provide a training in the key techniques of plant molecular biology. It is aimed at scientists wishing to expand their research to include this area. The course will cover the following techniques:
Gene fusion construction, DNA sequencing and computer analysis, plant nucleic acid isolation, southern and northern blotting, mRNA isolation, cDNA synthesis and cloning, PCR, oligonucleotide synthesis, pulse field electrophoresis, plant transformation using Agrobacterium, protoplast and particle bombardment techniques and analysis of reporter gene expression in transformed plants.
The practical work will be supplemented by lectures given by staff at Wye College on techniques and by visiting speakers on selected research topics. Speakers include: Dr. D. C. Baulcombe, Dr. M. W. Bevan, Dr. M. M. Burrell, Prof. R. B. Flavell, Dr. M. D. Gale, Dr C. M. Lazarus, Dr. G. Murphy.
Further particulars are obtainable from the course secretary: Mrs Sue Briant, Department of Biochemistry and Biological Sciences, Wye College, Wye, Ashford, Kent, TN25 5AH, U.K. Tel: 0233 812401 Fax: 0233 813320.

(6140)D

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CONFERENCES/MEETINGS

ROYAL SOCIETY MEETINGS MAY 1991

Thursday 16 and Friday 17 May: Joint Meeting with the American Philosophical Society. Symposia on **'New Biology', 'Science Policy'** and **'UK space science in the 1990s'**.

Wednesday 22 May 17.30: Evening Technology Lecture on **'New contraceptives: Victorian or Utopian?'** by Professor Carl Djerassi.

Thursday 23 May at 17.30: Lecture for the public on **'The triumph of the embryo'** by Professor L. Wolpert, F.R.S.*

Thursday 28 and Wednesday 29 May: Discussion Meeting with the Académie des Sciences de l'Institut de France *in Paris* on **'Cellular recognition and interaction'** organized by professor R. L. Gardner, F.R.S., Professor J.B. Gurdon, F.R.S. and Professor Anne E. Warner, F.R.S.

Thursday 30 and Friday 31 May: Discussion Meeting on **'Foraging strategies and natural diet in monkeys, apes and humans'** organized by Dr Elsie Widdowson, F.R.S. and Dr. A. Whiten.

Further information from: The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG (Tel: 071-839 5561, ext. 278/219*). (6169)S

THE ANNUAL HEPATITIS B VIRUS MEETING

The meeting co-chaired by Pierre Tiollais and William Robinson will be organized in the Pasteur Institute in Paris, from **September 9 to September 12, 1991.**

The topics of the meeting will include all aspects of the molecular biology of the hepatitis B virus.

Communications on Delta and hepatitis C viruses will also be accepted.

Information can be obtained from:
Madame Claude Volkerick, Service de la Documentation et des Relations Extérieures, Institut Pasteur, 25, rue du Docteur Roux, 75724 Paris Cédex 15, Tel: (33) 1.45.68.82.72. Fax: (33) 1.45.06.98.35. (W8430)S

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ANNOUNCEMENTS

NEDO

New Research and Development Projects of the New Energy and Industrial Technology Development Organization in Japanese Fiscal Year 1991

The New Energy and Industrial Technology Development Organization (NEDO), a semi-governmental organization established to implement the policies of the Ministry of International Trade and Industry of Japan, is pleased to announce its intention to undertake several new research and development projects in Japanese Fiscal Year (JFY) 1991 extending from April 1, 1991 to March 31, 1992. These new projects, which are undertaken as part of Japan's National Research and Development Program, Research and Development Program on Basic Technologies for Future Industries and Global Environment Technology Program, are outlined below.

A. OUTLINE OF EACH PROGRAM AND NEW PROJECTS FOR JFY 1991

(1) The National Research and Development Program

1) Outline of the program

Research and development concerning large-scale industrial technologies aiming at evolution of Industries, promotion of rational development and use of resources, prevention of industrial pollution and so on.

2) **Outline of new projects for JFY 1991**

a) Micromachine technology

Research and development on various types of basic technology and their integration available to the development of high performance micromachines which allow examination, diagnosis, repair and medical treatment in extremely narrow spaces.

(2) Research and Development Program on Basic Technologies for Future Industries

1) Outline of the program

Research and development concerning basic technologies for future industries which are highly innovative and whose research and development needs a long time period.

2) **Outline of new projects for JFY 1991**

a. Silicon-based polymers material

Research and development of technology for molecule design and synthesis to realize heat resistant, high rigidity and excellent electric characteristics for silicon-based polymers.

b. Production and utilization technology of complex carbohydrates.

Research and development of technology for synthesis both in vivo and in vitro and application of carbohydrates which play important roles such as activation of protein or lipid functions through conjugation with them.

c. Quantum functional devices

Research and development of quantum functional devices with super high-speed and multi-functions by developing technologies for ultra-micro structure formation and by utilizing quantum effects which occur in hyperfine structures.

(3) Global Environmental Technology Program

1) Outline of the program

Research and development of technology to be used as a countermeasure to overcome the global environmental problems which now face mankind.

2) Outline of new projects for JFY 1991

a. Environmentally friendly technology for the utilization of reusable metallic materials.

Research and development of technology for utilizing reusable metallic materials by utilizing oil-alternative energy, which is difficult to do at present, by means of introducing sophisticated processing, such as technology for making alloys of advanced-surface-processing technology.

b. Environmentally friendly technology for the production of hydrogen.

Research and development of technology for production of hydrogen utilizing microorganisms by using solar energy without consuming fossil fuels.

B. PROCEDURE FOR PARTICIPATION

(1) Official announcements calling for participants for each of the above projects will be made in the Ministry of International Trade and Industry's "Tsu-san-sho Koho", which is published daily in Tokyo. Copies of the announcement appearing in "Tsu-san-sho Koho" will also be distributed to OECD member country embassies in Japan.

(2) The timing for the above announcements has not as yet been fixed, except for the silicon-based polymers material project. (The announcement for the silicon-based polymers material project will be issued in the middle of June.) However, when the timing for the other announcements is fixed, NEDO will be able to notify those companies or organizations who inquire regarding such information.

(3) Those companies or organizations who wish to participate in a project are required to attend an explanatory meeting held by NEDO about two (2) weeks after the project announcement is issued. In case a responsible person from a company or organization is unable to attend this meeting, attendance by a substitute who is capable of explaining the contents of the meeting accurately is necessary.

(4) During the explanatory meeting, the contents of the new project and the application documents will be explained. Japanese will be the only language used during the meeting.

(5) The time period for submitting all applications is two months after the appearance of NEDO's official announcement in "Tsu-san-sho Koho".

(6) After the close of the application period, NEDO will notify all applicants the results of screening of their applications.

C. REMARKS

(1) The cost of project research and development will be borne by NEDO in accordance with its calculation of contract amounts, auditing methods concerning contract costs, and the terms and conditions which NEDO specifies.

(2) The results derived from NEDO-funded research and development projects shall be jointly owned by NEDO and the companies or organizations which conducted the research and development.

D. FURTHER INFORMATION

If you have any questions regarding this announcement, you may contact:

Mr Akito Sasaki, Manager, Contract Division, Accounting Department, New Energy and Industrial Technology Development Organization, Sunshine 60 Building, 28th Floor, 1-1 Higashi Ikebukuro, Toshima-ku, Tokyo.

Telephone: 03-3987-9322, Telefax: 03-5992-1184

(W8442)G

CONFERENCES/SEMINARS & SYMPOSIA (continued)

ENDOTHELIUM SYMPOSIUM

NATIONAL HEART & LUNG INSTITUTE
18, 19 & 20 NOVEMBER 1991

This is an exciting and fast moving area of research that has seen dramatic developments over the past two years, having far reaching consequences in many areas of medical research.

TOPICS

Atherosclerosis, reperfusion injury, solid tumour microcirculation, metastasis, angiogenesis, lung injury, nitric oxide, neuropeptides, endothelin and adhesion molecules.

PARTICIPANTS

Dr A Ager (Manchester), Professor E. E. Anggard (London), Dr S. A. Archer (Minneapolis, USA), Professor D. R. Blake (London), Professor S. R. Bloom (London), Dr S. D. Brain (London), Dr P. Collins (London), Professor J. Denekamp (London), Dr G Fearns (London), Dr J. Giddings (Cardiff), Dr D. Haskard (London), Dr K. Honn (Detroit, USA), Professor O Hudlicka (Birmingham), Professor J. MacDermot (London), Professor J. Martin (London), Dr S. Moncada (Beckenham), Dr C. Packard (Glasgow), Dr J. D. Pearson (London), Professor J. Polak (London), Professor U. S. Ryan (St Louis, USA), Professor T. A. Springer (Boston, USA), Professor Sir John Vane (London), Dr J. B. Warren (London), Dr C. Wegner (Ridgefield, USA), Professor A. H. Weston (Manchester) Dr F. M. Williams (London), Professor T. J. Williams (London), Professor N. Woolf (London).

ORGANISERS

Dr John Warren and Professor Timothy Williams, Applied Pharmacology Department, National Heart and Lung Institute.

FEE

For 3 days, including catering: £390. Academic rate £250

ENQUIRIES

Postgraduate Medical Centre, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY, tel: 071 351 8172, Fax: 071 376 3442. (6168)M

ANNOUNCING AN INTERNATIONAL IBC CONFERENCE

RHEUMATOID ARTHRITIS
Current Therapies and Future Directions
17th/18th June 1991
The Royal College of Physicians, London
by kind permission of the Treasurer

This conference will bring together a group of distinguished speakers to provide a comprehensive review of topics relevant to the current and future treatment of rheumatoid arthritis and allied disorders. These diseases continue to cause significant disability and morbidity and remain a challenge to scientists involved in both basic and clinical research. There will be a review of the epidemiology and socioeconomic impact of rheumatoid arthritis, and of current therapies.

The meeting should be of interest to all research scientists in academic institutions and from the pharmaceutical industry involved in work in immunology and inflammation, as well as to academic clinicians managing patients with rheumatoid arthritis and related disorders.

Chairman and Speakers:

Professor Theodore Pincus, Vanderbilt University, USA
Dr John Kirwan, Bristol Royal Infirmary, UK
Professor Gordon Duff, Royal Hallamshire Hospital, Sheffield, UK
Professor Gabriel Panayi, Guy's Hospital, London, UK
Dr Michael Bayliss, Kennedy Institute, London, UK
Professor Graham Russell, University of Sheffield, UK
Dr Alfred Walz, Theodor Kocher Institute, University of Berne, Switzerland
Dr Andrew MacKenzie, Sandoz Research Institute, Berne Ltd, Switzerland
Dr Russell Greig, SmithKline Beecham, Philadelphia, USA
Dr Rodger Macmillan, ICI, UK
Dr Allan Galloway, British Biotechnology Ltd, UK
Professor David Blake, The London Hospital, UK
Dr Dorian Haskard, Hammersmith Hospital, London, UK
Professor Tini Maini, Kennedy Institute, London, UK

For a full conference programme/registration details of the above event, please contact:

Catherine Barrett, IBC Technical Services Ltd
Bath House, 56 Holborn Viaduct,
London EC1A 2EX
Tel: 071-236 4080, Fax: 071-489 0849 (Intl +44)

(6145)C

THE CONVERGENCE OF

SCIENCE AND MYSTICISM

Einstein is reputed to have said that **"God does not play dice with the Universe".** Increasingly scientists speak of an integrated and ordered Cosmos mirroring the view of mystics and enshrined in the hermetic tradition. Helena Petrovna Blavatsky, Russian metaphysician, restored to the West this knowledge of the essential man. On the centenary of her death we reflect on her revolutionary **science of spirit** and how it has affected the way we think about human evolution and right relations with the planet and all fellow beings.

SPECIAL TRIBUTE Thursday 9th - Sunday 12th MAY 1991
at 50 Gloucester Place London W1 Details call 071 935 9261 or send sae to dept MP 91/1, **The Theosophical Society** at the above address

(6172)C

12th EUROPEAN DROSOPHILA RESEARCH CONFERENCE

MAINZ, GERMANY, SEPTEMBER 2-6 1991

This conference will be held on the Campus of the University of Mainz.

Registration will be on Monday September 2nd, with the scientific sessions starting at approximately 8.30 on Tuesday, ending at approximately 16.00 on Friday 6th September. There will be poster sessions in the afternoons and workshops in the evenings.

PLEASE NOTE:
Registrations and abstracts should be received as soon as possible, so please write to Judith Nicholls, Institute of Genetics, Johannes-Gutenberg University Mainz, Saarstr. 21, 6500 Mainz NOW for registration and Abstract forms.
FAX: 06131-395845 (W8432)C

COURSES continued

Royal Postgraduate Medical School

(University of London)

MODULE IN IMMUNOLOGY

A full-time five month postgraduate course commencing in February each year (next intake February 1992)

This course provides an advanced academic and laboratory training in modern cellular and molecular immunology, with emphasis on the interface between the basic and clinical aspects of the subject.

Course structure: The module comprises a five month taught course of lectures and practical classes.

Examinations: There are two written and one practical examination at the end of the module (July).

Entry requirements: Applications are invited from graduates in medicine, dentistry, basic and veterinary science who are interested in studying molecular and cellular immunology in a clinical context.

Qualifications obtained: The module may be taken alone as a postgraduate training in immunology. Alternatively it may be combined with a module in a second subject (chemical pathology, haematology, histopathology, microbiology) to obtain a University of London Diploma in Clinical Pathology.

Course organisers: Dr M A Ritter, H M Ladyman, N Reza

Application forms and further details from:

The School Registry
Royal Postgraduate Medical School
Du Cane Road
London W12 0NN

Telephone 081-740 3118 Fax 081-743 8995 (6118)D

COURSES continued

DEPARTMENT OF NEUROSCIENCE
Institute of Psychiatry
UNIVERSITY OF LONDON

MSc NEUROSCIENCE

MSc Neuroscience (one year, full time)

MSc (two years, part time)

INDIVIDUAL SHORT COURSES (one week, full time)

INTRODUCTORY COURSE

A multidisciplinary approach to neuroscience with emphasis on neurological and psychiatric disorders.

Neuroanatomy	Neuropathy
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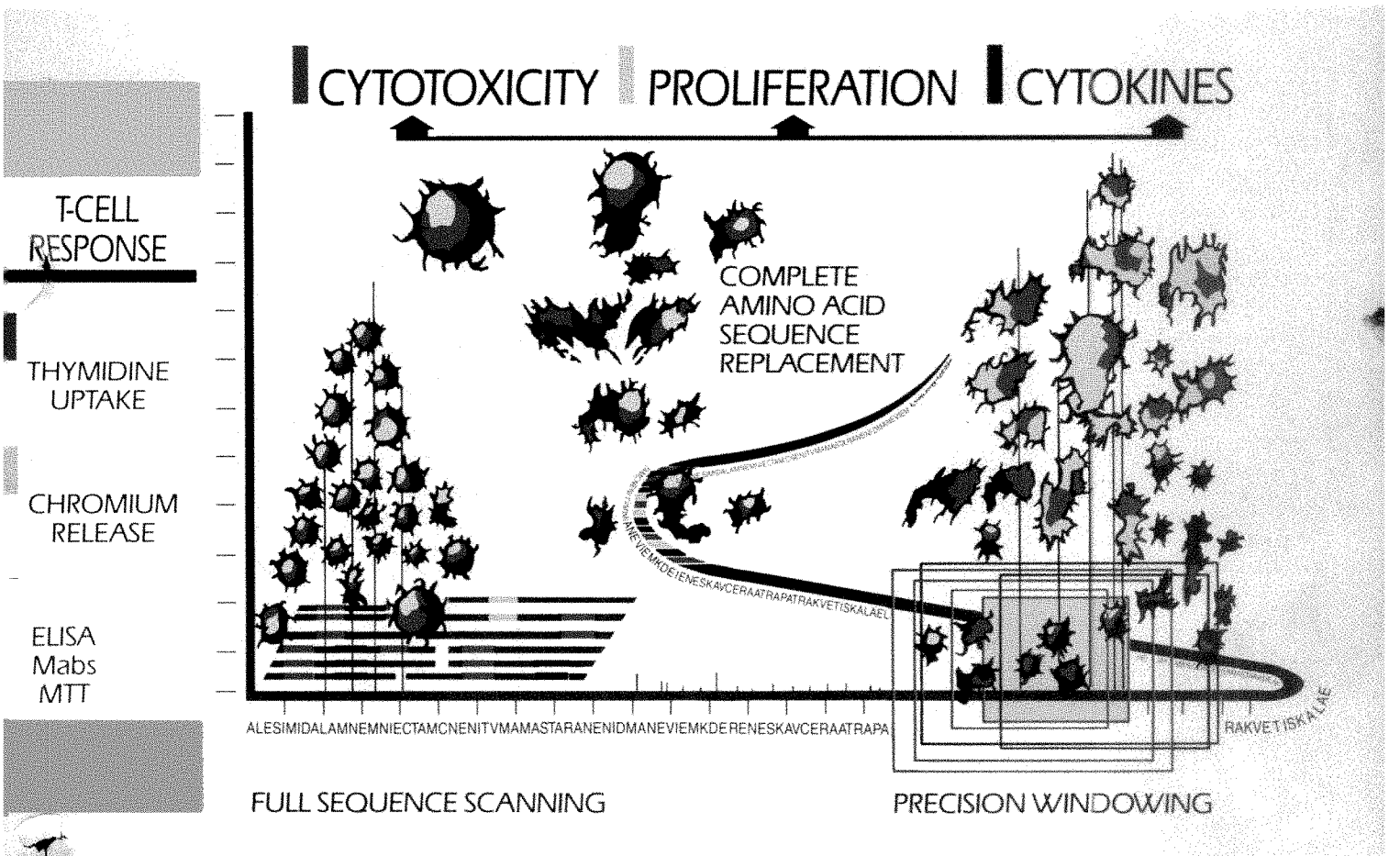

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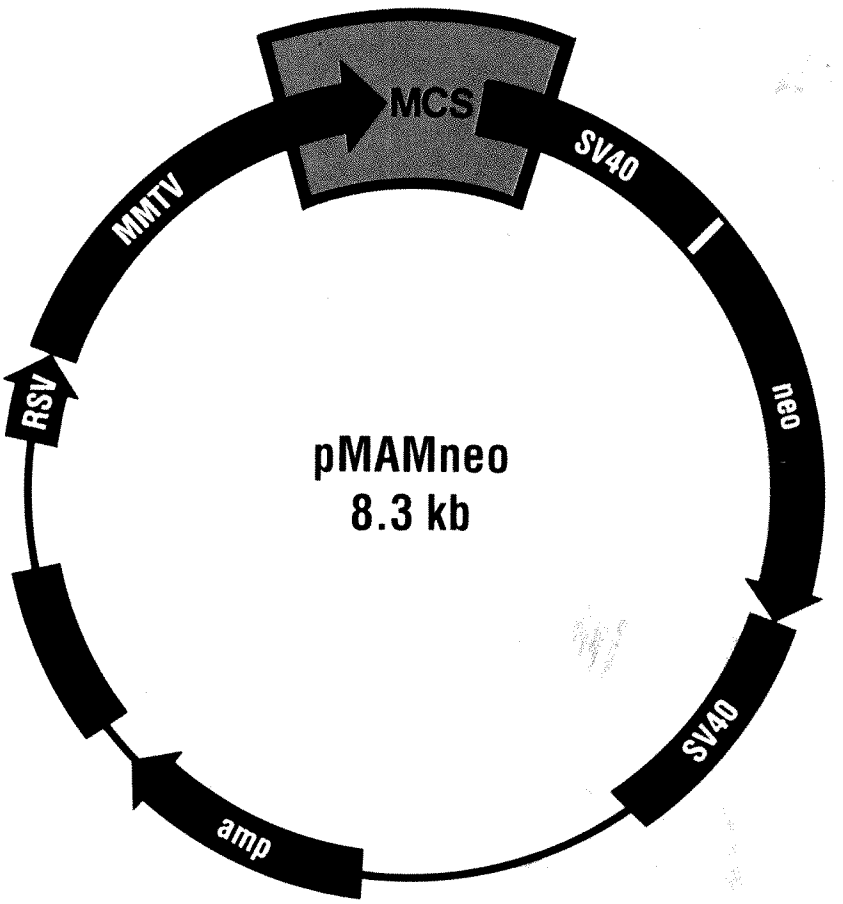

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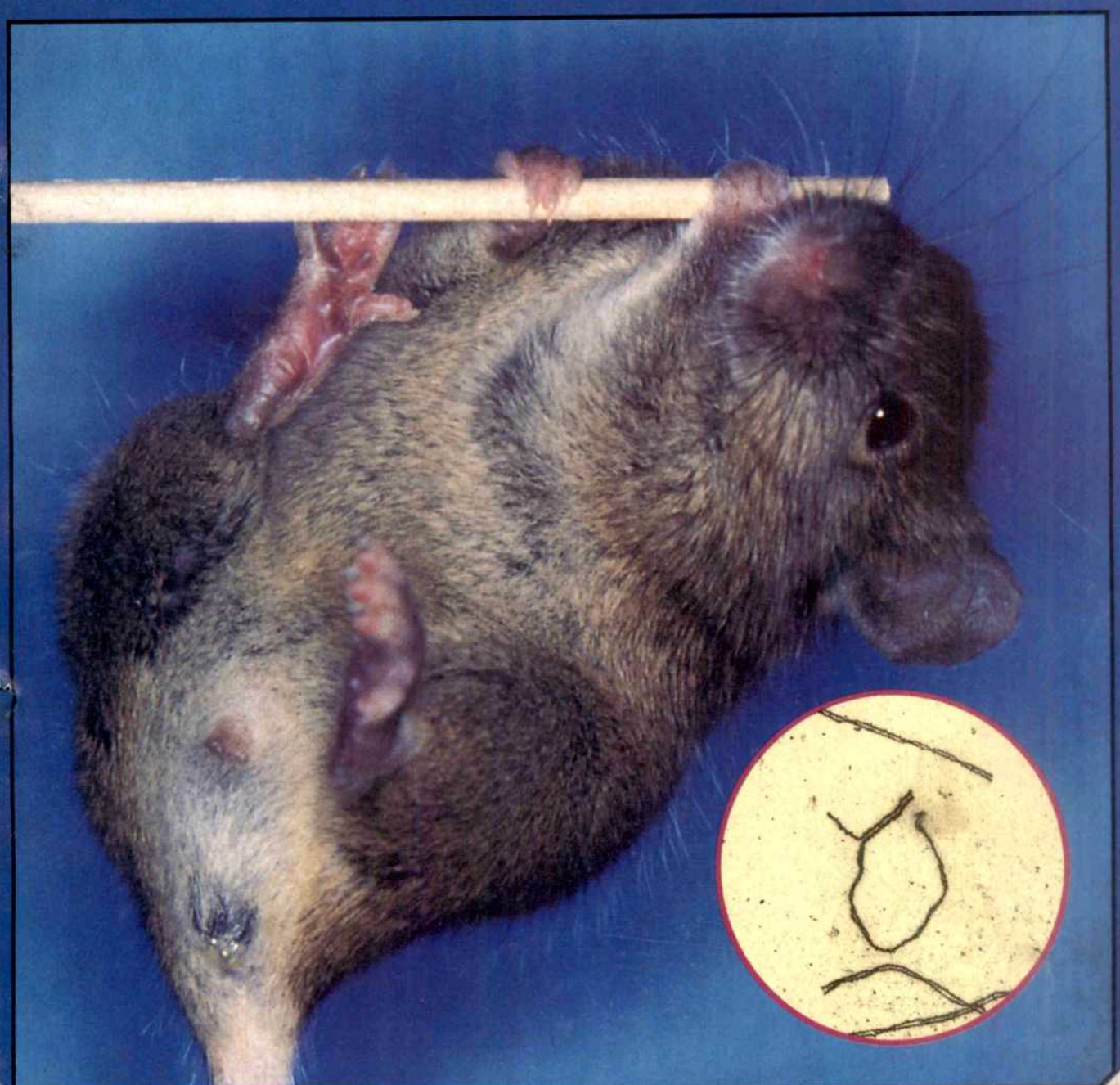

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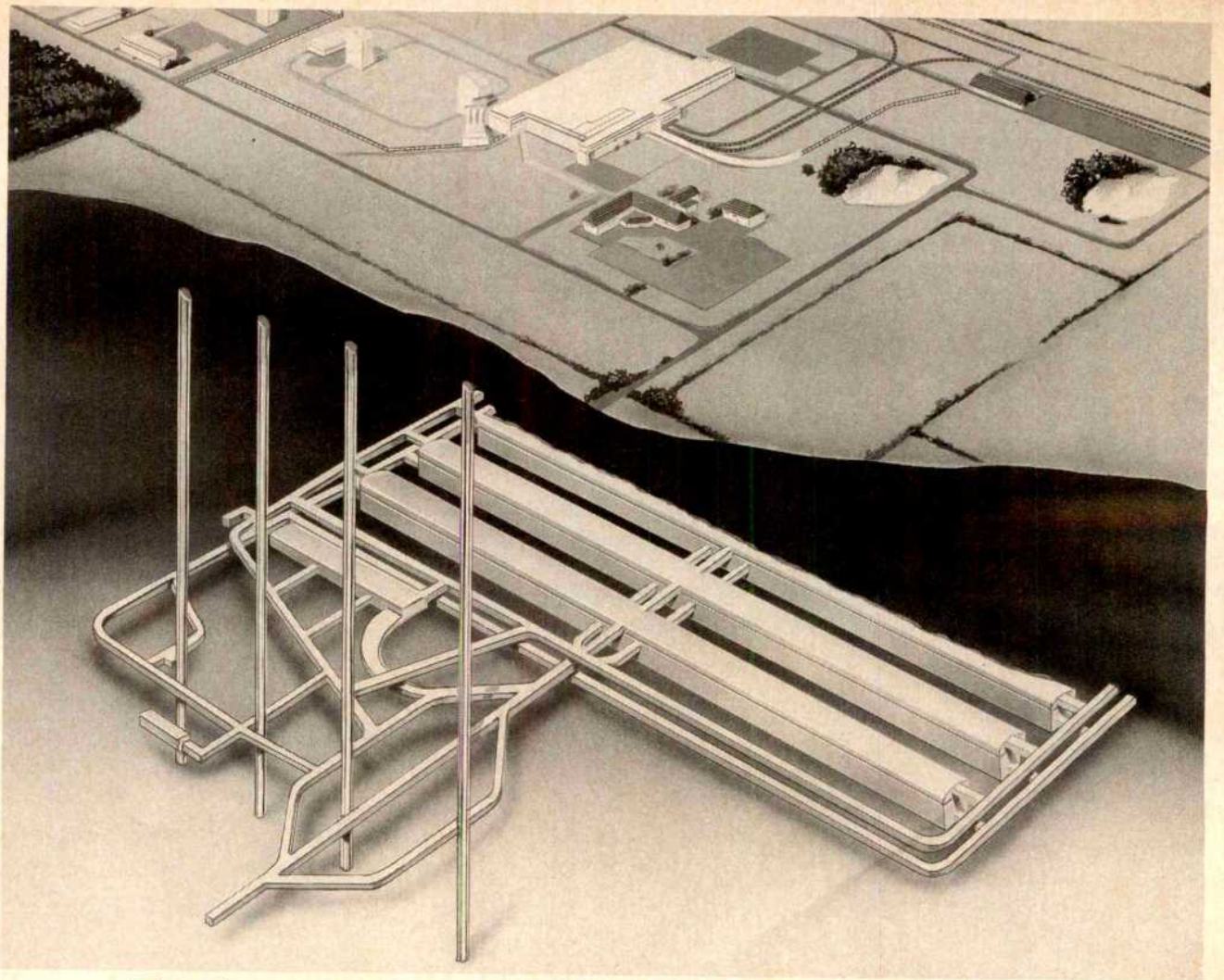

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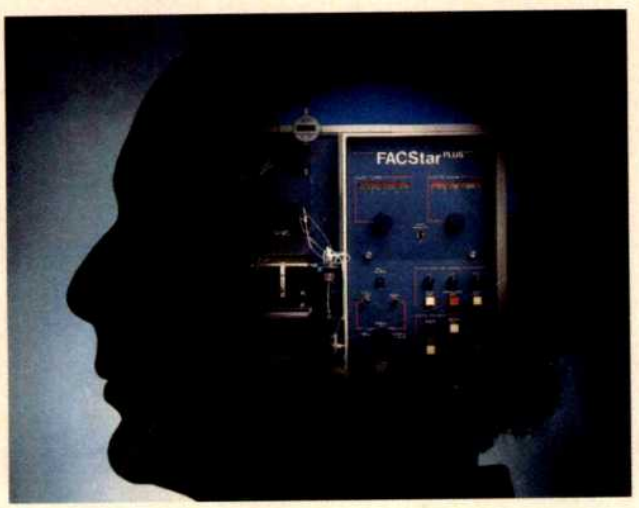

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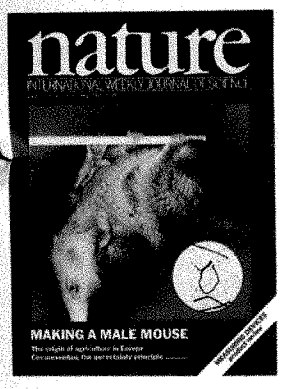

nature

9 May 1991
Vol. 351 Issue no.6322

◀ There is strong evidence that the murine *Sry* gene, normally near the pairing region of the chromosome, is identical to testis-determining factor. It is now shown (pages 117 and 96) that the *Sry* gene in a chromosomally female transgenic mouse (cover) induces normal testis development, and that *Sry* is the only Y chromosome gene necessary for male development. Inset, XY bivalent chromosomes, joined at point of homology (C. Tease, MRC Chilton).

THIS WEEK . . . THIS WEEK . . . THIS WEEK . . .

Fresh pastures
The 'wave of advance' model for the origins of European agriculture postulates that the techniques of agriculture spread across Europe from the Near East as part of a demographic process, where agriculture favours substantial population increase. Using blood protein data from modern human populations, Sokal *et al.* obtain genetic evidence to support this theory. Pages 143 and 97.

A certain something
The uncertainty principle makes it impossible to observe wave and particle behaviour simultaneously. But a new type of detector, constructed with the aid of modern quantum optics, provides a way around this obstacle in atom interferometers. Review Article, page 111.

History at fault
The fault in southern Greece thought to have been responsible for the destruction of Sparta in 464 BC has been identified on SPOT satellite images, and a recurrence time of about 3,000 years is predicted. Page 137.

AIDS pathogen
Alveolar macrophages are thought to be important in controlling infection by *Pneumocystis carinii*, an opportunistic pathogen that commonly strikes AIDS patients. The cellular receptors involved in recognizing the pathogen have hitherto been undefined, but new results show that the macrophage mannose receptor is necessary and sufficient for *P. carinii* binding and uptake. Page 155.

Oral tradition
The discovery of a new species of tetrapod from the Triassic of Virginia that has distinctive grooved teeth suggests that oral secretion of venom is a reptilian habit going back 200 million years. Page 141.

Nitrogen wastage
On page 135, Kuhlbusch *et al.* confirm that the clearance of large areas of tropical forests by burning is releasing significant amounts of nitrogen gas into the atmosphere, where it is not readily accessible to most living organisms. This loss of nutrient nitrogen could amount to as much as 50 per cent of global nitrogen fixation, with possible serious consequences for tropical ecosystems.

No culture
Many bacteria have proved recalcitrant to the standard techniques of bacteriological culture and so have never been fully characterized. Working with *Holospora obtusa*, an endosymbiont in a paramecium species, Amann *et al.* use PCR to achieve RNA sequence analysis of the still-uncultured microorganism. Page 161.

In the dark
The 'decaying dark matter' hypothesis attempts to explain the make-up of dark matter in the Universe based on the presence of massive neutrinos decaying into ultraviolet photons. But this hypothesis is effectively ruled out by new observations from the Hopkins ultraviolet telescope aboard the space shuttle Columbia. Pages 128 and 96.

Wave power
A system based on the Belousov–Zhabotinsky oscillatory reaction demonstrates coupled propagation of chemical waves across a membrane. The spiral waves on each side of the membrane are coupled by diffusion of neutral molecules from one side to the other. The system may have implications for the interpretation of intercellular communication and coupling of excitable media in biological systems. Pages 132 and 98.

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Quantum optical tests of complementarity
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1. Nielson, K. and Mathur, E.J. (1990) Strategies 3:17-19.
2. Nielson, K. and Mathur, E.J. Manuscript in preparation.
3. Nielson, K. and Mathur E.J. (1989) U.S. patents filed.
4. Mullis, K.B., and Faloona, F.A. (1987) Meth. Enzymol. 155:335-350.

Figure Legend: A photograph of a 1% agarose gel stained with ethidium bromide representing reaction products from PCR amplifications using the GeneAmp™ Kit† from Perkin-Elmer Cetus according to manufacturer's instructions. The reactions were conducted with (lanes 1 and 3) and without (lanes 2 and 4) the inclusion of 1 unit Perfect Match polymerase enhancer. Lanes 1 and 2 represent 100 ng of human genomic DNA amplified with two 26-mer primers separated by 1400 nucleotides. Lanes 3 and 4 represent 100 ng of mouse genomic DNA amplified with two 23-mer primers separated by 550 nucleotides.

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Ambion's reticulocyte lysate *in vitro* translation kit incorporates a remarkable 48% [^{35}S] Met using the control RNA. Linear incorporation for up to 2 hours.

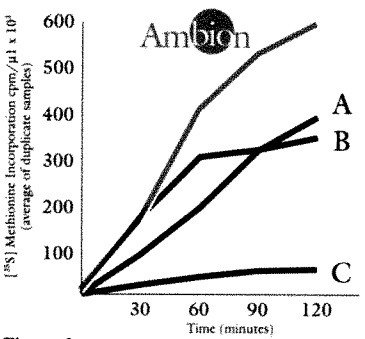

Figure 1
Comparison of Translational Activity of Ambion's Lysate with Lysates from Three Major Suppliers of Molecular Biology Reagents

TMV RNA at a concentration of 75 µg/ml was translated using each company's standard reaction conditions using 25 µl reaction volumes with 25 µCi of [^{35}S] methionine (1334 Ci/mmol).

2 Allows User Flexibility in Isotope Selection

Each Retic Lysate IVT Kit contains 4 different "Master Mixes" for capped and uncapped messages using either labeled Methionine or Leucine.

3 Efficiently Translates a wide variety of High MW Proteins (Fig. 2)

4 Low Background Incorp. (See –mRNA lane Fig. 2)

5 High Specificity for the Correct AUG Initiator Codon

(See *Nucleic Acids Research* 18:2828, 1990)

6 Translates Uncapped mRNAs (Fig. 3)

An Ambion exclusive. Alternative "Master Mixes" allow efficient translation of **uncapped** *in vitro* transcripts.

7 Convenient and Simple to Use

Brief Instructions for experienced users and Detailed Instructions for new users included in a comprehensive manual.

8 Expert Technical Support

Ambion's scientists have over 20 years experience in translation research.

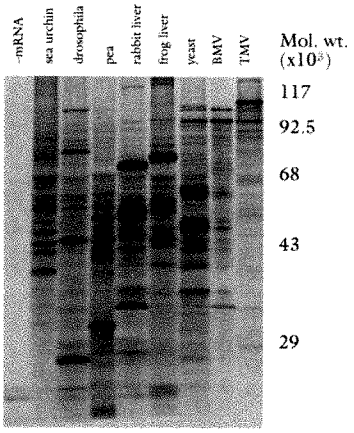

Figure 2
Efficient Translation of a Variety of High Molecular Weight Proteins

Total RNA from different sources was translated at 500 µg/ml for 60 minutes in 25 µl reactions using 12.5 µCi of [^{35}S] methionine (1200 Ci/mmol). Radiolabeled protein (50,000 cpm of assays containing total mRNA and 25,000 cpm of assays containing BMV and TMV RNA) was analyzed on a 10% polyacrylamide gel.

9 Consistent Lot-to-lot High Activity Lysate

Ambion's Retic Lysate IVT Kit consistency assured by rigorous QC and Production standards.

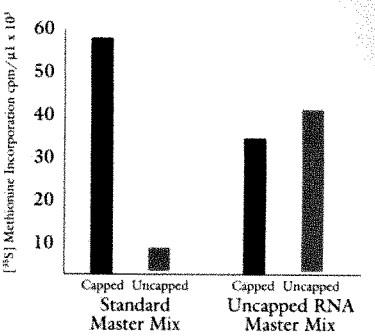

Figure 3
Comparison of Translation of Capped and Uncapped Globin Transcripts using Standard and Uncapped RNA "Master Mixes"

Capped and uncapped rabbit β-globin *in vitro* transcripts (6 µg/ml) were translated for 1 hour at 30°C in 25 µl reaction volumes using 25 µCi of [^{35}S] methionine (1334 Ci/mmol).

10 Accommodates Large Volumes of added mRNA & Label

Up to 28% of the final reaction volume is available for added mRNA and label.

11 Untreated Reticulocyte Lysate Kits Available

Used for translation elongation and initiation assays.

12 Great Value

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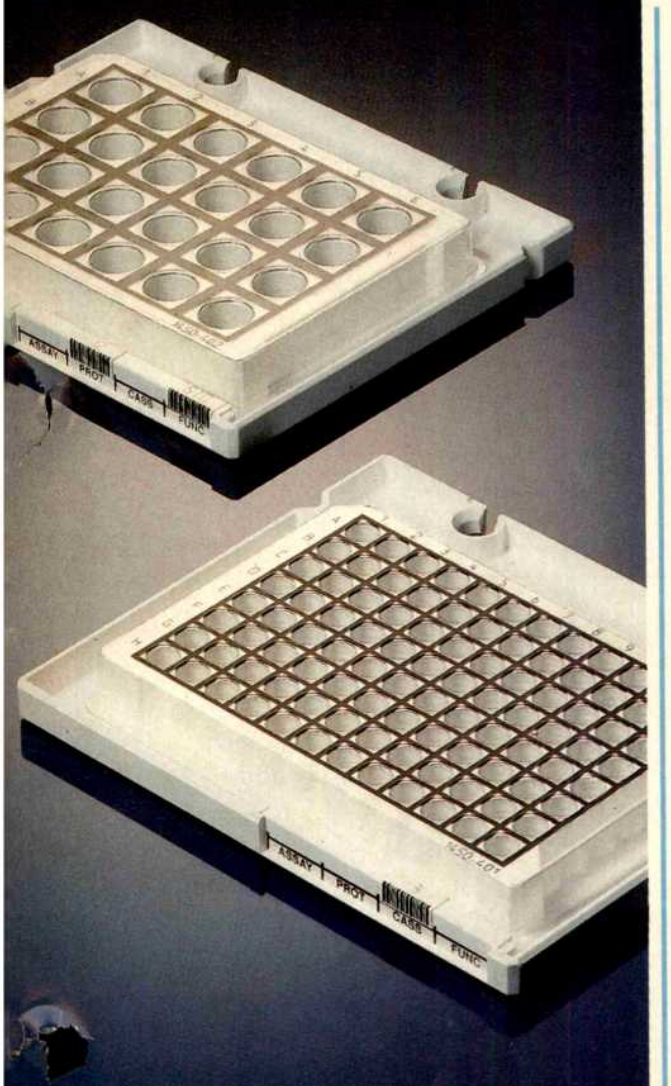

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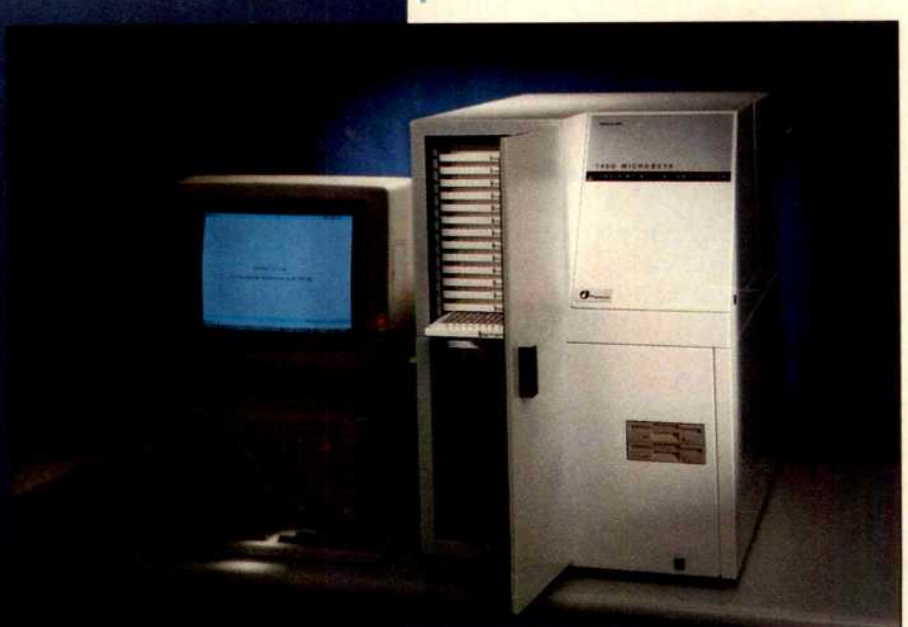

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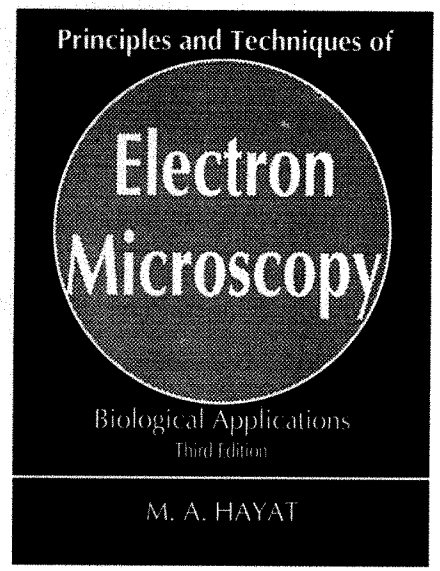

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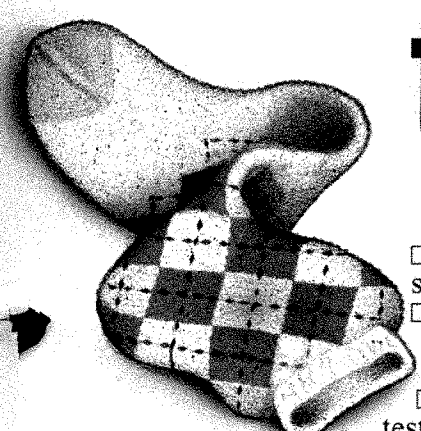

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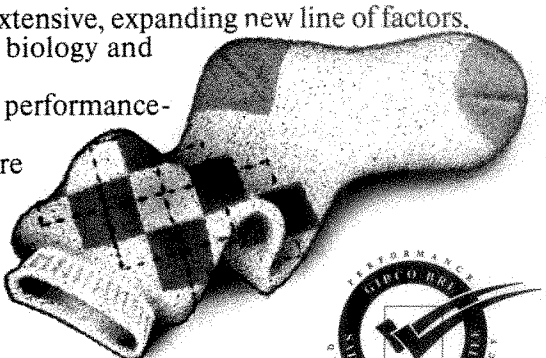

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UBIQUITIN AND PROTEIN DEGRADATION *June 9-14* Chairs: Alfred Goldberg, Harvard Med. Sch.; Richard Vierstra, Univ. of Wisconsin. **Enzymatic Systems for Ubiquitination and Deubiquitination I.** R. Vierstra, S. Prakash, S. Jentsch, C. Pickart, R.E. Cohen, A. Bachmair. **System for Protein Ubiquitination and Deubiquitination II.** S. Jentsch, K. Wilkinson, A. Varshavsky, A. Haas, T. Butt, Y.J. Yoo. **Marking Proteins for Ub-Dependent Degradation.** A. Ciechanover, D. Finley, R. Kulka, A. Varshavsky, V. Chau. **Regulation of Eukaryotic Cell Function by Protein Degradation.** M. Rechsteiner, P. Coffino, M. Kirschner or M. Glotzer, F. Luca, M. Hochstrasser, A. Hershko. **Proteasome and 1300kDa(26S) Proteolytic Complex.** A. Hershko, A.L. Goldberg, K. Tanaka, P. Kloetzel, K. Suzuki, J. Etlinger. **Regulation of Protein Degradation and Ubiquitin Expression in Pathological States.** A. Ichihara, L. Schwartz, S. Wing or R. Medina, J. Lowe, A. Taylor, K. Tanaka. **Proteolysis in Prokaryotes and Organelles.** A.L. Goldberg, M. Sherman, R. Sauer, M. Maurizi, C. Georgopoulos, F. Sherman, M. Edelman, M. Wettern. **Proteolysis in Lysosomes and Plant or Yeast Vacuoles.** D. Wolf, J.F. Dice, H-L. Chiang, R.J. Mayer, A. Ciechanover, D. Ho. **Degradation of Proteins in the Endoplasmic Reticulum.** J.F. Dice, J. Bonifacino, R. Sitia, G. van Bleck, T. Sommer or S. Jentsch, A. Smith.

NEUROIMMUNOLOGY *June 16-21* Chairs: Edward J. Goetzl, UCSF; Jean E. Merrill, UCLA. **Adhesion and Other Cell-Surface Molecules.** R.J. Milner, S.D. Rosen, B.A. Cunningham, D.R. Colman, J.L. Salzer, R.R. Lobb. **Poster Session: Mediators, Receptors, and Other Shared Molecules.** Chair: J. Majde. **Neural Circuits and Transmitters in Immunity.** D. Felten, K. Bulloch, E. Weihe, W. Maslinski, S. Felten, M. Badamchian. **Neuromediators and Cells in Immunity.** S. Leeman, B. Arnason, P. Mantyh, G.M. Jonakait, J. Weinstock, A. Kage, J. Luber-Narod. **Poster Session: Neuroendocrinology and Behavior.** Chair: N. Fabris. **Immunological Mediators of Neural Function.** J.E. Merrill, T. Roszman, M.L. Shin, W.F. Hickey, P. Paterson, E. Schreiber. **Receptors: Structure and Function.** M.S. O'Dorisio, S.P. Sreedharan, S.K. Dower, E.J. Blalock, S. Rosenzweig, L-Y. Yu-Lee. **Receptors: Transductional Pathways.** S. Gupta, C. Ottaway, P. Gardner, A. Nel, C. Ferris, R. Iyengar. **Neuroendocrine Effects in Immunity and Inflammation.** J. Martin, J. Lipton, A. Catania, J. Bienenstock, J. Levine, E. Sternberg. **Behavior and Responses to Stress.** J. Kiecolt-Glaser, J. Weiss, N. Cohen, R. Glaser, A.J. Dunn, B. Rabin. **Poster Session: Neuroimmune Diseases.** Chair: M. Yokoyama. **Viral Neuroimmunology.** R. Johnson, M. Buchmeier, R. Ahmed, L. Villarreal, B. Trapp, W.I. Lipkin, G. Koob, O. Narayan. **Anti-Synaptic and Paraneoplastic Autoimmune Neurologic Disorders.** V. Lennon, E. Lambert, S. Brimijoin, M. Solimena, K. Jaeckle, E. Lambert.

LYMPHOCYTES AND ANTIBODIES *June 23-28* Chairs: David Parker, Univ. of Massachusetts Med. Sch.; Laurie Glimcher, Harvard Sch. of Public Health. **Antigen Processing and Presentation.** P. Cresswell, E. Long, K. Rock, A. Townsend. **Mechanisms of Cellular Cytotoxicity.** P. Henkart, M. Kriegler, J. Ashwell. **Function and Dynamics of Primary and Memory Lymphocytes.** S. Swain, D. Gray, K. Hayakawa, S. Shaw. **Signals in Lymphocyte Activation.** E. Clark, J. Bolen, C. June, J. Cambier. **Patterns of Transcription in Lymphocyte Activation and Development.** L. Glimcher, K. Calame, D. Singer, J. Leiden. **Functions of Cytokines in Vivo.** R. Coffman, F. Finkelman, C. Jacob. **T Lymphocyte Development.** S. Hedrick, J.P. Allison, L. Matis. **Tolerance.** R. Schwartz, M. Julius, D. Parker. **Allorecognition and Graft Rejection.** A. Singer, H. Auchincloss, H. Winn, A. Rosenberg, K. Lafferty, S. Cobbold.

TRANSGENIC ANIMALS *June 30-July 5* Chairs: Jon W. Gordon, Mt. Sinai Med. Ctr.; George A. Scangos, Bayer AG, FRG. **Transgenic Mice in Immunology.** E. Robey, C. Breberich, K. Yamamura, J. Taurog, U. Storb, B. Hammer, N. First. **Insertional Mutagenesis.** S. Potter, R. Woychik, P. Tremblay, C. Stewart, P. Gruss, D. Wolgemuth, F. Ruddle. **Transgenic Mice and Aging.** D. Nerack, J. Mullens, J. Taylor, J. Majzoub, J. Breslow, G. Scangos, C. Markert. **Oncology/Human Disease Models.** L. Field, C. Knight, R. Manguez, T. Townes, E. Robertson, C. Stewart, G. Jay. **Genetic Engineering of Livestock.** J. Clark, S. Hughes, J. Gordon.

CELLULAR AND MOLECULAR GENETICS *July 7-12* Chairs: Kathryn Calame, Columbia Col. of Physicians and Surgeons; David Livingston, Harvard Med. Sch. **Cell Surface Receptors and Signal Transduction.** T. Roberts, M. Simon, C. Stiles, T. Parsons. **Steroid Receptors and Inducible Transcription Factors.** I. Verma, A. Berk, M. Groudine, R. Evans. **Transcriptional Controls in Cell Determination and Lineage Development.** K. Calame, M. Levine, G. Rosenfeld, B. Emerson. **Molecular Approaches to Mouse Development.** E. Robertson, L. Parada, P. Soriano, A. Bradley. **Transgenic Mice.** A. Berns, R. Jaenisch, R. Grosshedl, B. Gavin. **Human Sex Chromosomes and Sex Determination.** L. Shapiro. **Molecular Genetics of Human Diseases.** D. Housman. **Emerging Techniques for Mammalian Molecular Genetics.** D. Ward. **Recessive Oncogenes or Tumor Suppressors.** D. Livingston, S. Friend, J. Massagué, F. Spencer.

POSITIVE CONTROL OF TRANSCRIPTION INITIATION IN PROKARYOTES *July 14-19* Chair: Sankar Adhya, NCI, NIH; Co-organizers: S. Garges, G. Gussin, G. Storz. **Keynote Speaker:** R. Losick. **The Biochemistry of Transcription.** H. Bujard, M. Chamberlin, W. McClure, L. Rothman-Denes. **Sigma Factors.** C. Gross, P. Stragier, M. Susskind. **Two-Component Systems I.** S. Gottesman, B. Magasanik. **Two-Component Systems II.** J. Parkinson, H. Shinagawa, T. Silhavy. **Activator Paradigms.** R. Calender, J. Guest, W. Reznikoff, D. Wulff. **Contrasting Activators.** S. Long, M. Schwartz, M. Sekiguchi. **Protein-DNA Interactions.** R. Ebright, T. O'Halloran. **Role of DNA Structure.** H. Buc, D. Crothers, C. Higgins. **Activator-RNA Polymerase Interactions.** P. Geiduschek, G. Gussin, S. Kustu.

MOLECULAR MECHANISMS OF CARCINOGENESIS *July 21-26* Chairs: Michael W. Lieberman, Baylor Col. of Med.; Peter Howley, NCI, NIH. **Tumor Progression and Promotion.** H. Herschman, D. Hanahan, L. Liotta, G.T. Bowden. **Oncogenes and Gene Expression.** P. Vogt, B. Eisenman, I. Verma, M. Yaniv. **Oncogenes and Carcinogenesis.** M. Lieberman, D. Slamon, G. Van de Woude. **Viral Carcinogenesis.** P. Howley, F. Chisari, E. Kieff, G. Jay. **Growth Factors.** H. Moses, S. Aaronson, A. Roberts, C. Sherr. **Signal Transduction.** M. Weber, J. Brugge, T. Pawson, F. McCormick. **Tumor Suppressor Genes I.** E. Harlow, W. Cavenee, A. Levine, R. Weinberg. **Genetics of Cancer.** A. Knudson, J.C. Barrett, B. Seizinger, S. Barker. **Tumor Suppressor Genes II.** D. Livingston, W.H. Lee, S. Friend.

GENETIC RECOMBINATION AND GENOME REARRANGEMENTS *July 28 - August 2* Chairs: Richard Kolodner, Dana-Farber Cancer Inst.; Rodney Rothstein, Columbia Col. of Physicians and Surgeons. **Genome Rearrangements.** J. Roth. **Recombination Hotspots.** G. Smith. **Genetic Control of Recombination.** S. Roeder. **Physical Structures Involved in Recombination.** N. Grindley. **Recombination Enzymes From Procaryotes.** S. Kowalczykowski. **Recombination Enzymes from Eucaryotes.** L. Symington. **Transposition.** D. Sherratt. **Processing of Recombination Intermediates.** J. Haber. **Mechanisms of Recombination-Site Specific and General.** T. Petes.

LOW MOLECULAR WEIGHT GTP BINDING PROTEINS *August 4-9* Chairs: Gary M. Bokoch, Res. Inst. of Scripps Clinic; Channing J. Der, La Jolla Cancer Res. Fndn. **Overview Lectures: Evolution of the LMWG Family.** P. Chardin. **ras genes.** M. Barbacid. **ras structure.** S-H. Kim, A. Wittinghofer, S. Campbell-Burke. **Processing of LMWG.** J.E. Buss, J.F. Hancock, F. Tamanoi, J.L. Goldstein. **GTPase Activating Proteins for LMWG.** F. McCormick, J.B. Gibbs, J.A. Cooper. **rap/Krevl LMWG.** C.J. Der, M. Noda, Y. Takai, **YPT/SEC4/rab.** W.E. Balch, D. Gallwitz, P. Novick. **rho/rac LMWG.** A. Hall, J.R. Didsbury, S. Narumiya. **Miscellaneous LMWG.** R.A. Kahn, R. Cerione, D. Johnson, A. Tavitian. **Other Regulatory Molecules.** I.G. Macara, Y. Takai. **LMWG in Specialized Systems.** T.C. Sudhof, G.M. Bokoch.

RESEARCH CONFERENCES

MODULATION OF WOUND HEALING *August 11-16* Chairs: Thomas Hunt, UCSF; William Lindblad, Wayne State Univ.; H. Paul Ehrlich, Shrine Burn Unit of Massachusetts. **Cellular Response to Injury - Cytokines.** G. Grotendorst, C. Stiles, S. Aaronson, J.J. Oppenheim. **Angiogenesis and Endothelial Cell Responses.** G. Grotendorst, T. Maciag, D. Rifkin, P. DiCorleto. **Growth Factors in Repair I.** T. Hunt, G. Grotendorst, E.M. Spencer, K. Broadley. **Growth Factors in Repair II.** T. Hunt, N.A. Wright, E. Amento, S.R. Caughlin. **Collagen Biosynthetic Regulation.** W. Lindblad, J.C. Myers, G. Karsenty, T. Krieg. **Regulation of Collagen Deposition.** W. Lindblad, M.Z. Hussain, J.J. Jeffrey. **Metabolic Regulation of Wound Healing.** A. Barbul, M. Caldwell, N. Abumrad, S. Lowry. **Metabolic Regulation of Wound Healing.** A. Barbul, M. Robson, G. Warden. **Fetal Wound Healing.** I.K. Cohen, B. Mast, E. Hay, M.W.J. Ferguson.

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CHROMATIN AND TRANSCRIPTION *June 23-18* Chairs: Gordon Hager, NCI, NIH; Michael Grunstein, UCLA. **Structure of the Nucleosome and Histones.** T. Richmond, J. Thomas. **DNA Bending and Nucleosome Positioning.** R. Simpson, A. Travers, D. Crothers, A. Wolffe. **Chromatin Structure of Inducible Genes.** M. Beato, G. Hager, W. Horz. **Assembly of Chromatin.** B. Stillman, V. Jackson, R. Lasky. **Mechanisms of Repression.** M. Grunstein, R. Simpson. **Transcription from Chromatin.** B. Emerson, J. Workmann, C. Wu. **Active Domains.** G. Felsenfeld, F. Grosveld, A. Sippel. **Higher Order Structure I.** G. Hager. **Higher Order Structure II.** M. Bradbury.

OPIOIDS AND NEUROTRANSMITTERS IN ANALGESIA AND BEHAVIOR *June 30-July 5* Chairs: Thomas F. Burks, Univ. of Texas Health Sci. Ctr., Houston; Brian Cox, USUHS. **Nociceptive Systems.** T.F. Burks, W. Willis, T. Yaksh, H. Fields. **Opioid Analgesia in Animals and Man.** D. Mayer, A. Cowan, S. Holtzman, K. Foley. **Opioid Receptors.** H. Loh, P. Portoghese, V. Hruby, G. Pasternak. **Opioid Peptides.** A. Goldstein, L. Hersh, T. Davis, C. Chavkin. **Opioid-Monoamine Interactions in Spinal Cord.** J-M. Besson, M. Hamon, G. Wilcox, J. Sawynok. **Opioid - Neuropeptide Interactions.** G. Gebhart, F. Porreca, J. Fujimoto, A. Takemori. **Supraspinal Opioid-Neurotransmitter Interactions I.** A. Mulder, H. Proudfit, G. Aghajanian, J-C. Schwartz. **Keynote Speaker:** Louis Lasagna, **Clinical Studies on Opioids. Roundtable Discussion of Mechanisms of Analgesia.** B. Cox, E. Simon, A. Basbaum, A. Dray, C. Kornetsky.

PROTEIN KINASES *July 7-12* Chairs: Jackie Corbin, Vanderbilt Univ. Sch. of Med.; Michael Czech, Univ. of Massachusetts Med. Ctr. **Opening Keynote Address:** D.E. Koshland. **Calcium/Calmodulin Protein Kinases/Protein Kinase C.** T. Soderling, J. Stull, E. Severin. **Cyclic Nucleotide-Dependent Protein Kinases.** S. Taylor, J. Corbin, R. Tsien, L. Sowadski. **Tyrosine Protein Kinases.** M. Czech, R. Davis, W-H. Lee. **Gene Regulation by Protein Phosphorylation.** G.S. McKnight, M. Montminy, J. Dixon. **Ion Channels, Pumps, Membranes.** W. Catterall, M. Caron, P. Devreotes, M. Montal. **Cell Cycle Regulation by Protein Phosphorylation.** J. Maller, T. Hunt, J. Thorner, H. Piwnica-Worms. **Tyrosine Kinases.** T. Hunter, G. Carpenter, E. Krebs, N. Ahn. **Distinguished Lecture:** E. Fischer. **Novel Protein Kinases and Substrates.** D. Garbers, D. LaPorte, L. Johnson, G. Hardie.

SMOOTH MUSCLE *July 14-19* Chairs: James Stull, Southwestern Med. Ctr.; Cornelius van Breemen, Univ. of Miami. **Cell-Cell Communication.** D. Kreulen, K. Sanders, T. Cunnane. **Calcium Channels.** C. Benham, M. Nelson, H. Kuriyama. **Potassium Channels.** N. Standen, E. Stefani, T. Tomita. **Ion Pumps and Na/Ca Exchange.** R. Casteels, F. Wuytack, M. Blaustein. **Ca^{2+} Signaling Mechanisms.** C. van Breeman, F. Fay, T. Kitazawa. **Cyclic GMP Second Messenger System.** J. Corbin, D. Garbers, T. Lincoln. **Regulatory Properties of Contractile Proteins.** J. Stull, M. Ikebe, M. Walsh. **Modern Aspects of Mechanics.** D. Warshaw, J. Sellers, A. Arner. **Contractile Protein Expression: Gene Regulation and Adaptations.** C. Seidel, G. Gabbiani, R. Schwartz.

THE BIOLOGY AND CHEMISTRY OF VISION *July 21-26* Chairs: Bernard Fung, UCLA Sch. of Med.; John Lisman, Brandeis Univ. **Visual Pigments.** D. Oprian, G. Khorana, J. Nathans, R. Mathies. **Visual Transduction Mechanism - Biochemistry.** W. Baehr, Y.-K. Ho, V. Lipkin, D. Takemoto, W. Boenigk. **Visual Transduction Mechanism - Physiology.** P. Detwiler, D. Baylor, T.D. Lamb, R. Payne. **Light and Dark Adaptation.** G. Fain, R. Rando, H. Matthews, M.C. Cornwall, J. Schnapf. **Vertebrate Phototransduction and Molecular Genetics of Vision in Drosophila.** A. Fein, H. Saibil, E. Johnson, C. Montell, H. Matsumoto. **Photoreceptor Cell Biology.** J. Besharse, E. Burnside, R. Molday. **Common Motifs in Transduction Mechanisms.** G. Johnson, R. Reed, T.K. Harden. **Keynote Address:** H. Ripp. **Hereditary Diseases of the Retina.** M. LaVail, J. Turner, G. Aguirre. **Workshops: *In situ* Hybridization; Transgenic Mouse Methodology; Scanning Tunneling Microscopy.** D. Bok, M. Al-Ubaidi, N. Fisher.

ENDOTHELIUM AND CARDIOVASCULAR FUNCTION *July 28-August 2* Chairs: Paul Vanhoutte, Baylor Col. of Med.; Rudi Busse, Inst. of Applied Physiology, FRG; R. Wayne Alexander, Emory Univ. **Activation of Signal Transduction in Endothelial Cells.** R. Busse, D. Adams, P.E. DiCorleto, P. Davies, N. Flavahan, T. Brock. **Cytokines, Inflammation and the Vascular Wall.** P. Libby, M. Gimbrone, A. Mantovani, G. Zimmerman, J.S. Pober. **Blood-Endothelium Interactions.** E. Dejana, R. Cohen, D. Luskotoff, S. Silverstein, R. Rosenberg. **Endothelium-Derived Nitric Oxide.** S. Moncada, R.F. Furchgott, L. Ignarro, A. Mulsch, R. Palmer, F. Murad, D. Harrison. **Endothelium-Derived Contracting Factors.** P.M. Vanhoutte, G. Rubanyi, D. Harder, Z. Katusic, M. Yanagisawa, V. Miller, C. Boulanger. **Vascular Physiological Role of EDRF.** E. Bassenge, A. Zeiher, G. Kaley, J. Cooke, U. Pohl, W. Jackson. **Non-endothelial Origin of NO and Endothelin.** T. Masaki, V. Forsterman, V. Schini, M. Marletta, C. Romero, J. Billiar, S. Stojilkovic. **Endothelial Growth Factors.** W. Alexander, M. Majewski, T. Scott-Burden, T. Maciak, J. Folkman, M. Reidy. **Vascular Phenomena and Vascular Disease.** A. Herman, P. Henry, P. Ganz, T. Luscher, R.M. Robertson, D. Heistad, R. Levy.

GI TRACT IV: DEVELOPMENT AND REPAIR - CELLULAR AND MOLECULAR ASPECTS *August 4-9* Chairs: Michael Gershon, Columbia Col. of Physicians and Surgeons; Marian Neutra, Harvard Univ. **Stem Cells and Development (renewal of epithelium).** C. Potten, E. Birkenmeier, B. Ponder, N. LeDouarin. **Cell and Region-Specific Regulation of Intestinal Epithelial Gene Expression.** J. Gordon, R. MacDonald, A. Tyner, B. Ponder. **Models of Epithelial Differentiation.** A. Quaroni, E. Rodriquez-Boulan, D. Louvard, M. Rindler, M. Mooseker, M. Neutra. **Development and Maintenance of Polarized Epithelial Cell Function.** M. Caplan, M. Mooseker, J. Anderson, K. Mostov. **Endocytosis and Transcytosis in Mucosal Immunity.** M. Neutra, R. Rodewald, J. McGhee, J-P. Krahenbuhl, D. Powell, G. Castro. **Extracellular Modulation of GI Cell Differentiation.** J. Jamieson, V. Quaranta, P. Ekblom, L. Reid, L. Reichardt. **Cell Migration and Repair of the Epithelium.** J.L. Madara, L. Taylor, M. Sheetz, M. Beckerle, M. Sobel, L. Reichardt, V. Quaranta. **The Neural Crest and GI Development.** M. Gershon, N. LeDouarin, L. Reichardt, M. Bronner-Fraser, T. Rothman. **Intestinal Inflammation/Anaphylaxis and Neural Function.** D. Powell, M. Perdue, J. Wood, A. North, K. Barrett, G. Castro, H. Cooke, A. Suprenant. **Note: Two informal afternoon workshops will be added during the Conference. Topics: 1. Drugs and the bowel. 2. Applications of recent advances in GI research to inflammatory bowel disease.**

CYTOKINES AND LIPID MEDIATORS AS REGULATORS OF CELL FUNCTION *August 11-16* Chairs: Patrick Y-K. Wong, New York Med. Col.; Robert Lewis, Syntex Corp. **Keynote Address:** K.F. Austen. **Cytokines, Phospholipases, G-Proteins and Cell Activation.** P. Vadas, E. Dennis, E. Lapetina, A.R. Morrison, M. Clerk. **Cytokines and Phospholipase A^2.** D. Morgan, W. Pruzanski and R.W. Egan, R. Kramer, R. Crowl. **Cytokines, Oxygen Radicals and Tissue Injury.** M.S. Wolin, A.M. Lefer, R. Ramwell, G. Feuerstein, M. Palladino, F.F. Sun. **Lipid Mediators and Cell - Cell Interaction.** C.R.P. Asciak, C. Serhan, R.C. Murphy, F. Fitzpatrick, P. Borgeat, R. Soberman. **Cytokines and Lipid Mediators in Immunoregulation.** K.C. Arai, R.A. Lewis, S. Gillis, S. Dower, J.W. Chao, K. Smith, T.G. Nagabhushan. **Regulation of Arachidonic Acid Oxygenation.** K.K. Wu, K.F. Austen, R.R. Gorman, J. Evans, T. Yoshimoto. **Cytokines and Platelet Activating Factor.** J. Wallach, S. Prescott, W. Hsueh, J.M. Mencia, B.B. Vargaftig. **Cytokines, Lipid Mediators, and Inflammation.** R. Hedquist, S. Yamamoto, T. Lee, S.E. Dahlen, K.V. Honn, M. Foegh. **Cytokines, Eicosanoids and Receptor Signalling.** S. Mong, G. Fitzgerald, P. Halushka, P. Y-K. Wong, J.M. Dyer.

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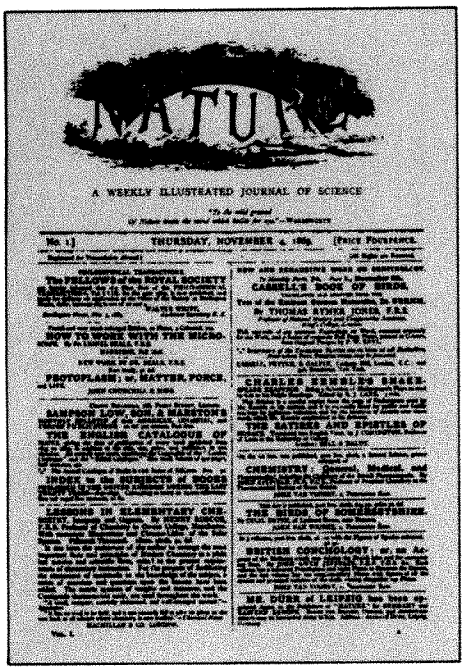
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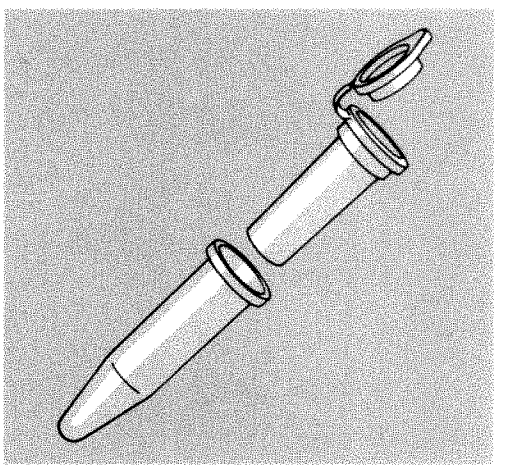

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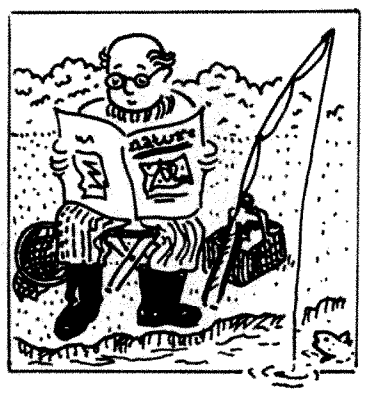

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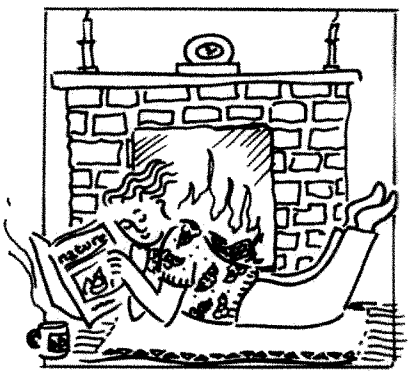

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The end of the Baltimore saga

One of the most corrosive disputes of recent years in the research community should be ended with the open acknowledgment of the error of an excess trust by the principal in the case.

Dr David Baltimore, president of Rockefeller University in New York, has done the decent thing. In his statement on page 94, he acknowledges that he was wrong to have defended the data of his colleague Dr Thereza Imanishi-Kari in the face of mounting doubts. He has also implicitly acknowledged that his truculence before the Dingell Committee two years ago was, to say the least of it, inappropriate; now he says that government has a legitimate right to inquire into the uses made of public money in research. Above all, he offers an apology to Dr Margot O'Toole, the postdoctoral fellow in Imanishi-Kari's laboratory who first became suspicious of the data published in 1986. That will not erase her painful experiences, but it is a handsome apology.

So what happens now? Has Baltimore said enough to bring an end to the process of inquiry that has consumed the past four years of many people's lives? There is some unfinished business, but one thing is clear: Baltimore has said enough to restore his own reputation as a fine scientist, a man of public spirit and a potentially superb and certainly imaginative president of an outstanding and distinctive research university. Some among his colleagues may be tempted to use this public acknowledgement of error by their leader as an excuse for furthering their own narrow causes, but they should instead reflect on what Baltimore's ingenuity may eventually accomplish for their institution. To make an error may reflect on a person's judgement, but to confess it in the circumstances in which Baltimore now finds himself is a mark of courage. He deserves a break.

Opportunity

Others will be less fortunate. The National Institutes of Health (NIH) have yet to say what will happen to Imanishi-Kari, against whom the charge of fabricating data had been laid. She is an able scientist, if a poor record keeper. What the Office of Scientific Integrity demonstrated last month is that data in a notebook submitted to the Dingell Committee had been cobbled together from different and irrelevant sources, but the status of the originally published data is still not clear. In one sense, it no longer matters, for the article concerned has been withdrawn. But both NIH and the Dingell Committee will want to know how this state of affairs arose. It is especially important that Imanishi-Kari should be given an opportunity to put her case. It is unlikely that this case would have been so prominent had it not been for the association with Baltimore.

The question will also arise of how an article now acknowledged to be unsound could have been published in a journal holding to rigorous standards of peer review. Baltimore himself says that his defence of his co-author was sustained by his "trust in the efficacy of the peer review process". But reviewers cannot tell whether data in an article submitted for publication are false except when there are internal inconsistencies or flagrant discrepancies with data already published elsewhere. Reviewers, rather, judge whether conclusions follow legitimately from the data offered in their support. In the disputed article, suggestive of an unexpected interaction between endogenous and transplanted genes in a mouse, the conclusions did not ring out with the clarity of a bell. The reviewers might well have asked that the work should have been confirmed before publication — and would probably have been scorned. It is therefore to be hoped that one outcome of this case will be a more proper recognition (especially in the Congress) of the limitations of peer review.

Pitfalls

So how can fraud be avoided in the future? Baltimore promises to "participate actively" in working out new guidelines for dealing with allegations of fraud and for protecting those who make them. Fine. But what can be done? This and other cases point to the importance of full and contemporaneous laboratory notebooks. More important still, they demonstrate the pitfalls for institutions that embark on investigations in the belief that allegations of misconduct are almost by definition unfounded. Whistleblowers have a lonely and thankless task which they are unlikely to take on lightly. Institutions have a duty to be more diligent than has usually been the case. They will thus safeguard not merely their own reputations, but also their independence. The mere existence of the Office of Scientific Integrity is a mark of how much they have lost already.

The incidence of misconduct also needs attention. By now there is ample evidence that the driving force lies in the competitive character of academic research, especially in the United States. People cut corners or even fabricate data so as to simulate success and earn the rewards it brings — another grant, perhaps, or even a promotion. The same competitiveness has also made US biomedical research into the most successful scientific enterprise anywhere. The problem is how best to abate the evil consequences of competition without losing the benefits. That, more usefully than the development of further guidelines for the conduct of investigations, is the issue into which Baltimore should now put his energies. □

Market heats up for US biotech companies

■ Record amounts of capital raised
■ New products pave the way

Washington

AFTER four years of uninspired performance, biotechnology stocks have once again become hot properties on Wall Street. Since the beginning of the year, 20 biotechnology companies have raised a staggering $915 million of new capital in the public markets — a figure that already exceeds the amount raised during the whole of 1986, when the last wave of financial offerings flowed out from the industry.

Financial analysts say that the renewed interest in biotechnology stocks stems from several factors. As more products are introduced into the marketplace and as companies begin to make meaningful profits, investors are beginning to view the technology as having less risk. In particular, the commercial success of erythropoietin (EPO) has shown that genetically engineered drugs have the potential to be quite lucrative. And a couple of favourable patent decisions have reassured investors that companies will be able to reap the profits of successful invention.

The upshot is that the biotechnology industry is "at the right place at the right time", says Linda Miller, stock analyst with Paine Webber.

The lion's share of the money raised through public stock offerings has been snapped up by top-tier, well established companies, but six little-known firms have amassed more than $200 million by going public. Almost half of this amount — $99 million — was raised by Regeneron Pharmaceuticals Inc., a neuroscience company established only in 1988.

Regeneron's research focus is the development of biotechnology-based compounds for the treatment of neurological disorders, such as Alzheimer's disease and Parkinson's disease. What makes the company's ability to raise $99 million so striking, says John Girton, a stock analyst with Van Kasper, is that "the development and commercialization of any products is years away".

The proceeds from that stock offering, which are mostly earmarked for research and development, will enable Regeneron to maintain its current and planned operations well into 1994.

The success of Regeneron and other biotech companies in raising capital demonstrates a renewed faith on the part of investors in the potential of fundamental biotechnology research. Early enthusiasm for biotechnology products on Wall Street was dampened when tissue plasminogen activator (TPA), hailed as biotechnology's flagship product, fell short of sales targets. Data from two comparative trials of clot-busting agents indicated that TPA is no more effective than streptokinase, a drug produced by more conventional means at one-tenth of the price.

Interest revived last year, however, when EPO, another biotech drug, had worldwide sales of $630 million. The drug, which stimulates the body to produce more of its own red blood cells, is used to treat anaemia in patients on kidney dialysis as well as HIV-infected individuals taking AZT. Sales of EPO are expected to 'mushroom' as other therapeutic uses for EPO are discovered.

More biotech drugs are in the pipeline. Since the beginning of the year, both Amgen Inc. and Immunex Corporation have received US Food and Drug Administration (FDA) approval for their colony stimulating factors; at least eight more biotech drugs should be approved in 1991. After years of anticipation, this steady stream of drug approvals means that more companies will have a source of revenue and will thus be making the transition to profitability.

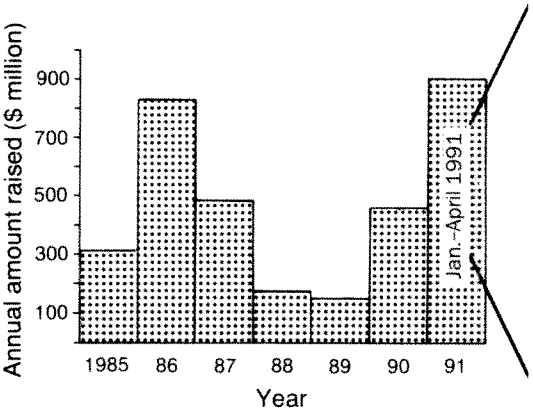
Adding to the optimism about biotechnology's future are the resolutions of two important patent disputes earlier this year.

Amgen won a resounding and unexpected victory over Genetics Institute for the US patent rights to EPO, and Cetus Corporation successfully fended off a challenge by Du Pont over the polymerase chain reaction. These court decisions should allow investors better to assess the patent risks surrounding a potential biotechnology investment.

Amgen's victory over Genetics Institute in its fight over patent rights to EPO, which has left Amgen with a guaranteed US market, is one of two factors that has caused the company's stock to more than quadruple in value in the past 12 months. The other is the recent FDA approval of Amgen's second potential blockbuster biotech drug, granulocyte-colony stimulating factor.

The growing interest in biotech companies has pushed executive salaries quite high, according to a recent survey by the management consulting firm of William M. Mercer of Boston. Of the 26 biotechnology companies surveyed, chief executive officers received an average of $608,300 in total compensation, including stock incentives. This, Mercer says, is 71 per cent more than the average of $355,300 earned by CEOs of high-tech companies, and 114 per cent more than earned by CEOs in industry as a whole.

"Good biotech executives are hard to find," says Girton at Van Kasper. Finding a person with the right mix of a knowledge of the industry and an entrepreneurial spirit can make "the difference between success and failure for these companies," he says.



	ISSUER	STOCK OFFERING ($ MILLION)
1	Ionics	32.6
2	Cygnus Therapeutic Systems	27.0
3	Phoenix Advanced Technology	2.0
4	Chiron	115.0
5	Immunex	107.0
6	California Biotechnology	36.6
7	Immunogen	30.4
8	Seismed Instruments	4.1
9	Somanetics	7.2
10	Gensia Pharmaceuticals	33.4
11	Genzyme	97.6
12	Synergen	86.3
13	Aphton	7.0
14	Regeneron Pharmaceuticals	99.0
15	XOMA	79.5
16	Enzon	33.4
17	Vestar	15.0
18	Cephalon	59.4
19	Calgene	15.0
20	Immune Response	27.5
		914.8

Source: IDD Information Services, New York

US biotechnology companies have had tremendous success this year in public stock offerings, raising twice as much in the first four months as in all of 1990.

Not every biotech company is having an easy time finding success. Genetics Institute, for instance, has been beset with problems. Not only was it on the losing end in its struggle with Amgen over EPO, but a court decision over US patent rights to TPA has also gone against the company. Now Genetics Institute and its partners are locked out of the US market for both drugs. Just two weeks ago, Gabriel Schmergel, president and chief executive officer of Genetics Institute, announced his intention to withdraw a proposed common stock offering because of "the recent decline in our common stock share price".

Although most analysts believe that the window of opportunity will be open for some time, most agree that only those companies that merit financing will have access to the market. Having had 10 years of experience with the biotechnology industry, investors now have the ability to ask "a lot of tough questions". "Selectivity is still the watchword," Miller says. **Diane Gershon**

GERMAN RESEARCH

Ray of hope for the East

Munich

RESEARCHERS in eastern Germany received an important vote of confidence two weeks ago from the Fraunhofer Gesellschaft, a Munich-based organization that supports and directs scientific research at several dozen institutes in western Germany. The Gesellschaft announced that it will create 19 research institutes and outstations in the former East Germany by merging existing groups from a variety of institutions, thus making it more likely that these groups will survive the turmoil brought on by merging science in the east and west.

By pledging DM500 million (about $281 million) to the 19 institutes, the Gesellschaft, a non-profit research organization with ties to government and industry, is making the biggest single commitment to research in eastern Germany since German unification.

Although it will affect only certain fields of applied research, the Gesellschaft's support is encouraging for researchers who had begun to wonder whether the West would see any value at all in their work.

The decision is also seen as a step towards rebuilding the collapsing industrial base of eastern Germany. The small- and medium-sized companies, known as the *Mittelstand*, which form the backbone of Germany's industrial economy, often depend on having publicly funded research and development activity nearby.

Now, with the Gesellschaft's action, "there will be a higher density of Fraunhofer institutes in eastern Germany than there is in western Germany," says Wilhelm Krull of the science advisory council Wissenschaftsrat, which had a hand in deciding which groups to take over. By setting up a dense network of application-orientated research centres, Krull says, Fraunhofer Gesellschaft is providing a foundation upon which new or revamped industry can build.

Between 1992 and the end of 1994, the Gesellschaft will distribute the DM500 million among the 19 institutions — ten new institutes and nine outstations of existing western institutes. In addition, the Gesellschaft seeks an additional DM70 million to set up even more institutes. The move is expected to save the jobs of at least 950 researchers and supporting staff.

By moving quickly and decisively into eastern Germany, Fraunhofer Gesellschaft has put pressure on the German government to raise its level of commitment to the eastern part of the country. The governments in Bonn and the *Länder* (states) will have to pour in at least another DM300 million just to renovate the dilapidated buildings in which the institutes are housed and make them fit for modern research. Even more money will be required for modern research equipment, especially computers compatible with those used in industry. "We are counting on Bonn and the *Länder* to help," says Gesellschaft spokesman Alexander Rothhämel.

All 19 institutions will be composed of researchers who worked in East German institutes, universities or industry. Like the general working population, eastern German research workers face an uncertain future now that their organizations, especially the East German Academy of Sciences, are being shut down or reorganized.

Among those fortunate enough to be selected are a number of groups that used to work at the academy, which in the years before unification had been shifting its emphasis increasingly towards applied research. Because of export restrictions on high technology, the academy and East German industry were forced to develop on their own technologies that were widely available in the West. In the process, eastern Germans developed a technological resourcefulness that is impressive to their Western colleagues. The Gesellschaft decision is a concrete indication that they have not been working for naught.

If the new institutes are to survive past their initial three-year period of support by the Gesellschaft, they will have to generate revenue by doing contract research for industry. The Gesellschaft has said it expects the institutes to cover 25 per cent of their costs through contract research. The 38 Fraunhofer Institutes in the west generally cover about 80 per cent of their costs through outside contracts. But the Gesellschaft recognizes that it will take time for the eastern institutes to discover the most profitable fields of research.

The list of groups and institutes to be taken over includes no surprises; among them are the Academy institutes thought to have the best chance of surviving in a united Germany. The significance of the decision by Fraunhofer is that related groups will be allowed to keep working together rather than having to take their chances on the open market. One example is the central institute for cybernetics and information processing in East Berlin, where, in the transition phase before unification, clever programmers had already found western contracts to augment their income. Other fields on the list include solid state physics, ceramics, polymer chemistry and applied optics.

The Gesellschaft left itself two ways out in case the research services that its eastern institutes provide prove to be superfluous. After three years, the Gesellschaft may either close the institutes or move some of the researchers to related western institutes.

Because a move to the west goes against Bonn's philosophy about unifying German research — namely, to keep as much of eastern research as possible in the east — a Gesellschaft spokesman says he expects such a westward shift to occur in only a small number of cases.

Steven Dickman

SPACE STATION

Auditors fret, NASA persists

Washington

CONTROVERSY over the price of the planned international space station — the one constant in the embattled project's tumultuous decade of design — has struck again. A new US government accounting audit shows that the space station may eventually cost as much as $40,000 million more than expected. Coupled with an emerging consensus from the scientific community that, as now designed, the space station will be of minimal scientific use, the news may set the stage for the toughest battle yet in Congress over the future of the project.

The audit, by the congressional General Accounting Office (GAO), found that NASA (the National Aeronautics and Space Administration) had not included the costs of such items as a crew-escape module and a artificial-gravity centrifuge in its estimates to Congress. NASA also neglected to include the costs of maintaining and operating the station once it is completed, GAO said.

"Once these costs are added together," GAO comptroller general Charles Bowsher testified at a congressional hearing last week, "what we actually have is at least a $118 billion program — about $40 billion to achieve permanent occupancy and about $78 billion to keep the station operational between 2000 and 2027." In defence of the project, senior NASA officials dismissed the audit findings as a matter of differing accounting methodologies and called for an end to such reviews. "It's time to come to a decision," testified NASA administrator Richard Truly. "Let's give it to the engineers and let them go build it."

Since 1984, NASA has redesigned the space station eight times to adapt to new technical, financial and congressional demands. In the process, the station has shrunk from an eight-person, six-module, 125-kW design whose development was expected to cost $8,000 million to a four-person, three-module, 56-kW design expected to cost $16,900 million to develop and $13,000 million to deploy. In development alone, NASA's expects to pay twice its 1984 estimate for half as much station. And if GAO is right, the real cost may be even higher — $10,000 million more in the development phase and $34,000 million over NASA's estimates during the operating phase.

The history of such space station controversies has been consistent. Each new study has revealed that the project will probably cost more and deliver less science than previously promised. Each time, the grim figures have prompted new calls for the project's cancellation. Yet, so far, the US champions of the project have been able successfully to argue that it should still be built.

Christopher Anderson

SERC RESEARCH GRANTS

Award now, pay later

London

UNIVERSITIES that win grants from the UK Science and Engineering Research Council (SERC) may be forced to borrow money to buy equipment and hire research assistants, under a new SERC policy that is designed to relieve the council's immediate financial difficulties.

The reason is that SERC, in its latest round of grants, has imposed strict limits on what percentage of a grant can be spent in its first two years. The details vary from project to project, but some researchers will be given only 15 per cent of a three year grant to spend in the first year. In some cases, SERC intends to hold back more than 50 per cent of a grant until the final year of the project.

This, however, is precisely the opposite of the way that many researchers need their grants to be structured. Grant recipients often need to spend a large proportion of their awards in the first two years of a project, particularly when expensive items of equipment must be purchased. SERC's policy, therefore, will force universities to borrow money to cover the start-up costs of research projects, says David Thomas, industrial liaison officer at Imperial College, London, who is also responsible for Imperial's income from the research councils.

Thomas fears that the policy could "change the criteria by which grants are awarded". Rather than the best projects being funded, he believes grants may go to the richer universities, or those that are prepared to slip into debt. Jim Reed, industrial liaison officer at the University of Surrey, is now conducting a survey of his colleagues around the country to gauge the extent of the difficulties the new policy will bring for the universities.

SERC officials say that the spending limits will help relieve SERC's well publicized cash flow problems (see *Nature* **349**, 551; 14 February 1991), but add that there is "no intention to place any additional financial burden on the universities".

The research council is operating under the assumption that its finances will be in a better state two years from now, and that it will then have no problem honouring the grants in full.

SERC chairman Sir Mark Richmond is determined to protect research grant spending from further cuts. But even with the new policy of spending limits for the first two years of new research projects, SERC expects to award only 50 per cent of the usual number of new research grants this year.

Peter Aldhous

UK RESEARCH FUNDING

Reform may cut grant awards

London

The British research councils would have to cut their grant spending in the universities by as much as 12 per cent if the government goes through with a plan to reform British university research funding, according to a confidential report presented last week to Education and Science Secretary Kenneth Clarke.

From the 1992–93 academic year, the government aims to transfer £100 million a year to the research councils from the research budget of the Universities Funding Council (UFC). The idea is that in the future the research councils will pay the overhead costs (administrative costs, telephone bills and the like) of research projects they support. The universities would then be responsible only for the salaries of permanent academic staff and the costs of running university buildings.

But the new report, prepared by the Committee of Vice-Chancellors and Principals (CVCP) and the research councils, says that £100 million is inadequate to cover the overhead costs of research council-funded projects. The CVCP calculates that £138 million is needed; the research councils put the figure at £149 million. Their analysis is based on the costs of a sample of research projects in ten British universities.

If the research councils are to pay the overhead costs of each project they support but are given only an extra £100 million to do so, the report argues, they will have little option but to award fewer research grants.

The proposed transfer has not created a new problem — it has merely made more evident the universities' assertion that they have not been getting paid enough for research for some time. "It is clear that some universities have been paying for the direct costs of projects supported by research council grants to a far greater extent ... than has been recognized," the CVCP concludes in the report. And Derek Roberts, provost of University College London, argues that the underfunding of research costs in the universities is some £100 million more than suggested by the research councils: the report excluded the cost of mainframe computing, and did not consider projects funded by charities.

The report, which was commissioned by the government, should put pressure on the Department of Education and Science (DES) to provide more money for research. But Clarke is not expected to abandon the planned transfer, which has already been delayed by one year. The DES is convinced that the transfer will improve the administration of research spending.

Peter Aldhous

ANTARCTIC ENVIRONMENT

Mining ban in the air

London

MINING in Antarctica would be banned for at least 50 years if the governments of the Antarctic Treaty nations sign a draft protocol on Antarctic environmental protection produced last week at a meeting in Madrid. But it is not clear whether the Bush Administration in the United States will approve the version of the treaty agreed to by its negotiators, and a rejection of the draft could yet prevent consensus on the divisive mining issue when Antarctic Treaty nations meet next month.

The draft text would ban mining and oil exploration for the next half a century. Even after then, the ban could be relaxed only if all of the present 26 consultative parties to the Antarctic Treaty agree — generally seen as an unlikely prospect.

The protocol has pleased environmentalist groups and those governments, led by Australia and France, that were pushing for a permanent ban. James Martin Jones, from the World Wide Fund for Nature, says agreement on the draft "would give us what could be described as an indefinite ban."

Before the meeting, a group of nations led by Britain and the United States was expected to ensure that the option of mining in the future was kept open. But the group was weakened by a surprise policy reversal by Japan, which was previously a strong supporter of the mining option (see *Nature* **348**, 570; 1990). The Japanese delegation announced at the start of the meeting that Japan would support a ban that could be lifted only by consensus.

Observers in Madrid now expect the United States to be the only possible obstacle to agreement on the draft protocol. The draft is understood to have gone well beyond the negotiating brief given to Curtis Bohlen, head of the US State Department delegation.

The US administration has until a second meeting in Madrid, beginning on 17 June, to consider the draft. State Department officials last week refused to comment.

Britain, however, is expected to accept the draft. The UK Foreign Office has said that consensus on the mining issue is its number one priority, and rejection of a draft supported by the vast majority of Antarctic nations would be politically embarrassing.

Aside from the mining ban, the draft environmental protocol contains new provisions to assess the environmental impact of proposed research projects in Antarctica. Antarctic scientists had feared that a new regime of environmental impact assessment would place undue restrictions on Antarctic research. Under the new draft, the environmental impact of some research projects will have to be assessed. But this will be carried out by national authorities, and is not expected to place obstacles in the way of research.

Peter Aldhous

ENDANGERED SPECIES

Emerging virus threat

Washington

In January, a small group of golden lion tamarins waited in a holding facility at the National Zoological Park for a trip to Brazil. Eleven of the foot-long monkeys were to be released into the wild as part of the zoo's reintroduction campaign for endangered species. Then, three days before the tamarins were scheduled to leave, zoo pathologists discovered that one of the monkeys posed a potential threat to South American wildlife. The tamarin was carrying antibodies against the callitrichid hepatitis virus (CHV), an infectious organism that has recently struck primate populations in nearly a dozen US zoos. Indigenous to Old World primates, the virus has never been seen in the New World outside captivity.

The pathologists, who already knew the tamarin to have been exposed to CHV, learned at the last minute that the virus could be transmitted to other animals through rodents — a discovery that made it much more threatening. If the monkey had carried CHV into the wilds of Brazil, where the virus has never been seen, it might have spread to other primates and other susceptible species.

The consequences would be impossible to predict. The virus might have died out with no effect. But the pathologists had to consider the worst-case scenario — that the virus might have caused a plague that would devastate some vulnerable species.

The experience with the tamarins highlights a dilemma facing reintroduction programmes. Although the value of importing endangered species back into their natural habitats is unquestioned, some researchers are now beginning to reexamine the safety of such campaigns.

The danger arises from what are known as 'emerging viruses'. Like AIDS and hepatitis, emerging viruses are previously non-threatening viruses that can decimate new populations by acquiring fresh hosts and vectors — often with the unknowing help of humans who introduce new species into virgin environments. Canine distemper, for example, has wiped out the black-footed ferret in the wild and was most probably introduced by domestic dogs.

National Zoological Park

Does this golden lion tamarin carry an 'emerging virus'?

Reintroducing an existing species into its own original environment can also risk an epidemic, especially when those animals have been exposed to the cocktail of viruses that proliferates in a zoo. In the case of the hooded crane, a probably foreign herpes virus has slowed reintroduction and further endangered the last of the surviving wild population.

"It's a very serious potential problem," says Benjamin Beck, associate director for biological programmes at the National Zoo. "It's only because of our advanced diagnostic facilities that we were able to catch this virus. Who knows what else is going through?" In response, some countries have essentially prohibited reintroduction by instituting such strict import regulations that even animals only recently removed from the wild cannot be returned.

Yet most researchers believe that they have little choice but to go ahead, albeit cautiously. Devra Kleinman, the National Zoo's assistant director for research, says, "To maintain maximum genetic diversity we're going to have to inject animals into the wild. Plague is a risk, but it's a risk we feel we have to take."

Six other US zoo programmes are participating in reintroduction and breeding projects for tamarins, among some 55 other endangered species that are now being reintroduced. To minimize the danger of an epidemic, zoo staff quarantine tamarins for several months before reintroduction, during which time the animals are examined for parasite eggs, viruses and genetic problems. "We check for the things we know," says zoo pathologist Richard Montali. "Anything that we can treat, we treat. Any animals we have questions about, don't go." Overall, about 15 per cent of the tamarins are disqualified for one reason or another.

After the animals are released in Brazil, seven zoo specialists observe them every day over a six-month quarantine period in a remote area away from wild tamarins. If the researchers spot signs of a disease outbreak, they can trap and remove the monkeys within hours.

So far, zoo staff have recorded no transmission of disease from the 75 tamarins that have been reintroduced to any of the local animals. But as Beck points out, "We're only systematically observing tamarins. Who knows what has happened to the beetle population?"

The potential problem can be traced back to the zoos where animals are kept before reintroduction. With hundreds of species in close proximity, and insects and rodents often in plentiful supply as intermediate vectors, zoo animals are exposed to a host of foreign pathogens and parasites. Some of the viruses affect the animals themselves, others may simply use them as a carrier.

In the case of CHV, pathologists believe that rodents carried the organism from Old World to New World primates at a zoo, probably through the baby mice (known as 'pinkies') with which the tamarins are fed. Another tamarin disease, caused by a parasite found in bush babies (small simians that are also kept at the National Zoo) appears to use insects as its vector. Cockroaches, which are as common in the tamarins' zoo quarters as they are in nearby Washington apartments, are a favourite tamarin snack and the chief suspect in that case.

"The diseases are evolving faster than we are identifying them," Kleinman says. "The parasites are constantly changing to keep one step ahead of the hosts."

Such a moving target makes complete screening impossible in reintroduction programmes. New viruses are going to get through, if they have not already. Zoo officials hope that they can screen out the worst of the risks, but they admit that they are gambling on good luck and quarantines.

"When we decided in the beginning to go ahead with the reintroductions, we didn't know exactly what the risks were, and we still don't," Kleinman says.

The researchers point out that other human activities, from agriculture to ecotourism, are also bringing new diseases to wild populations. "We're exposing Brazil to less risk by exposing it to our tamarins than we are by exposing it to ourselves," Beck argues.

While that may be true for the country at large, something like an infected tamarin can be the ultimate nightmare for the species that the researchers are trying to save. Unlike the zoo-bred animals, the wild populations have not had generations in which to become immune to the foreign virus.

Most researchers agree that the best solution in many cases would be *in situ* conservation — the breeding and management of endangered species within their natural environment. If the animals can be protected in areas near where they are naturally found, the risk of their being exposed to new diseases is minimized.

But conservation programmes are expensive, and most are funded by zoos, which in turn depend on public support. Although reintroduction programmes may be intellectually attractive, people are unlikely to support the effort if they cannot see the animals.

So most zoos, strapped for cash, continue to run their reintroduction programmes in the high visibility of captivity. They screen for what diseases they can, and hope for the best with what they miss. "It's an area where we're playing God," says Michael Hutchens, director for conservation and science for the American Association of Zoological Parks and Aquaria. "But we don't have a lot of choice."

Christopher Anderson

JAPANESE GOVERNMENT LABORATORIES

MITI to shake up institutes

Tokyo

THE first major reorganization of Japanese government laboratories in decades is now being planned by the Agency of Industrial Science and Technology (AIST), an arm of the powerful Ministry of International Trade and Industry (MITI). The plan to shuffle several research institutes in Tsukuba science city is intended to inject new life and greater flexibility into the government research system.

Tsukuba science city, which was created in the 1970s by uprooting government research institutes from Tokyo and transplanting them to the rice paddy fields of Ibaraki Prefecture north of the capital city, has come to life in the past few years with a sudden influx of private sector research institutes (see *Nature* **345**, 378; 1990). But while some government research laboratories are flourishing in the new environment, others are bogged down in bureaucracy and an organizational structure that dates back to the beginning of this century. The latter category includes the four AIST institutes targeted for reorganization, which one leading optoelectronics researcher in Tsukuba calls "dinosaurs".

Those institutes are the National Chemical Laboratory for Industry, the Fermentation Research Institute, the Research Institute for Polymers and Textiles and the Industrial Products Research Institute. They have a total of more than 500 researchers and a combined annual budget of over ¥8,000 million ($60 million).

If the AIST plan is put into effect, the four will be combined into two new institutes, one specializing in life sciences and the other in materials science.

In addition, AIST will establish an entirely new research centre for interdisciplinary research that will focus on nanotechnology and 'holistic science'.

Researchers in the life sciences and materials science are now scattered among various divisions of the four AIST institutes that are huddled together in one section of Tsukuba. The current plan is to bring all the life scientists into the present Fermentation Research Institute and a new building to be constructed nearby, while putting the material scientists in the National Chemical Laboratory and the adjacent Research Institute of Polymers and Textiles, which will also get a new wing. The Industrial Products Research Institute, meanwhile, will be converted into the new interdisciplinary research centre, although a section of it will be set aside for the life scientists.

AIST officials expect the total outlay for new construction work to amount to ¥4,500 million ($33 million) over the next three years. The agency has already allocated ¥410 million ($3 million) for this fiscal year with which to begin construction of the new building.

The most radical element of the plan is the proposed new interdisciplinary research centre, which has several aspects that depart from normal Japanese practice.

Instead of having permanent staff employed on a lifetime basis — as is now the case in all government research laboratories — researchers will be assigned to the centre to work on projects for periods of three to five years. At the end of that time, the projects will undergo "severe" review, says Masayoshi Hamada of AIST, who is in charge of the reorganization.

Researchers will be drawn not only from AIST laboratories but also from private industry, universities and overseas. Non-Japanese scientists will be involved in the planning stages of the projects, rather than just being invited as 'guests'. Hamada expects the centre to have a total complement of about 100 researchers.

In many respects, the new centre will resemble the Research Center for Advanced Science and Technology (RCAST) of Tokyo University and the Frontier Research Program (FRP) of the Institute of Physical and Chemical Research (RIKEN), based in the outskirts of Tokyo. RCAST and FRP are two successful anomalies recently created in the government research sector. RCAST has a number of chairs funded by private industry. RIKEN is a 'special corporation' (*tokushuhojin*) and, although it is affiliated to the Science and Technology Agency, it has much greater freedom and flexibility to establish programmes such as FRP than do ordinary government research laboratories.

The AIST centre will concentrate on two seemingly contradictory themes. One group of researchers working in an 'atom factory' will examine and manipulate biological and inorganic material at the level of atoms and molecules with scanning tunnelling microscopes, atomic force microscopes and other devices for working at the nanometre level. A second group, working in the opposite direction from this reductionist approach, will study whole organisms, societies and technologies holistically.

AIST still faces one hurdle before its new plan can be implemented. The reorganization has to be approved by the Management and Coordination Agency, which, under a policy of fiscal restraint implemented in the early 1980s, is steadily trimming the number of government employees, including researchers, to try to cope with the national government's chronic debt. AIST will submit a formal proposal to the agency in late August and a decision will be made at the end of the year. But AIST officials say they are confident of success because their plan does not require any increase in the number of permanent government employees.

David Swinbanks

Moving to nanotechnology

Tokyo

AIST's decision to establish an 'atom factory' in a new research centre in Tsukuba (see above) is part of a massive move by MITI to promote research into nanotechnology.

This fiscal year, MITI will launch a 'next generation' (*jiseidai*) project to develop quantum dot and quantum well devices, with a budget of about ¥5,000 million ($40 million) over ten years. Several major electronics manufacturers are expected to join the project this summer (see *Nature* **349**, 449; 1991).

And the *jiseidai* project is only the beginning. MITI officials are now sketching out a large-scale project that, with the collaboration of dozens of companies, will plough hundreds of millions of dollars into the development of 'ångström technology'.

This proposal is a strong candidate for inclusion in MITI's budget request for fiscal year 1992, to be submitted at the end of August. The eventual intention, says Masayoshi Hamada of AIST, is to link the proposed atom factory in Tsukuba with the ångström technology project. **D.S.**

US NATIONAL ACADEMY

Thier plans to leave Institute of Medicine

Washington

SAMUEL Thier, who presided over the US Institute of Medicine (IOM) for six years of unprecedented growth, is leaving to become president of Brandeis University. He said last week that he plans to leave IOM by 1 October, and that the institute is in the process of assembling a search committee to find a new president.

Part of the National Academy of Sciences, IOM is an independent body of scientists who provide the federal government, Congress and others with advice on biomedical issues. During Thier's tenure, the amount of IOM contract studies grew from some $3 million to $14 million, and it now has nearly 30 studies under way. In addition, an endowment was created and grew to the current level of $20 million.

Asked why he is leaving for academic life at a time when IOM is doing well and the university community is facing cutbacks and stalled growth, Thier said that he is "intrigued by challenge". Brandeis, as the smallest and the youngest of the members of the research orientated Association of American Universities, "is an opportunity to do some interesting experiments in reorganization", he said.

Before joining IOM in 1985, Thier was chairman of the department of internal medicine at the Yale University School of Medicine. Christopher Anderson

Turmoil in European biology

Lennart Philipson

A power struggle between various European organizations is hindering attempts to expand basic biological research in Europe. A remedy to this situation must involve both scientists and top politicians.

AMONG the natural sciences, biology and especially molecular biology, is growing most rapidly and shows great promise for the future. Biotechnology, fed by the discoveries of basic molecular biology, is rapidly gaining momentum. Molecular biology also appears to be a necessary ingredient in medicine, agriculture and attempts to correct major faults already introduced into the environment, such as overfertilization of the soil, contamination of air and water and exhaustion of energy resources.

The Human Genome Project and related genome projects, together with the approaching economic unification of Europe, have led to an increase in proposals for new projects in biology to be organized and supported at the European level. These same proposals have, unfortunately, also revealed the animosity and struggle for power within and between the different European organizations involved in funding biological research. It has become abundantly clear that we need — but do not have — a coordinated European policy for the support of basic and applied research in biology.

The players

The players in the field are numerous. The organization with the most substantial funds for European collaborative projects is the European Commission (EC). It concentrates foremost on applied projects directed from the top which are often initiated without proper consultation with the scientific community. Through the single European Act, the EC has its own revenue which is constantly growing, mainly as a result of the reduction in the subsidies of agricultural products. At the political level, EC proposals are subject to review and approval by the European Parliament and the Conference of Science Ministers.

The European Science Foundation (ESF) depends mostly on the resources provided by the research councils which are its constituent organizations. This inevitably leads to a conflict between the truly European and the national interest. Research council money provided to the ESF cannot be spent nationally, with the result that the ESF's budget is kept at a minimum level.

The European Molecular Biology Organization (EMBO) and the European Molecular Biology Laboratory (EMBL), both devoted to basic research in molecular biology, receive their funds directly from the 17 and 15 member states respectively. The national delegates are at a much lower political level than those at the EC and, in one member state, the funds for the European Molecular Biology Conference (EMBC) and EMBL are in competition with resources for national projects.

HUGO (the Human Genome Organization), a global organization aimed specifically at promoting the Human Genome Project, has so far only support from private foundations which, of course, leads to a weaker position in the political arena.

EMBO and the EMBL have for several years pointed out, in vain, the need for a stronger European base in basic molecular biology. They have put forward proposals which involve requests for additional money for more pre- and postdoctoral fellowships, for a programme of research groups and even for additional EMBLs. This package of proposals was strongly supported by a majority of the EMBO membership. A necessary expansion of the EMBL DNA Data Library into an independent European Bioinformatics Institute was not included in that package, but it has received support from an ESF report on the Human Genome Project and from an EC-funded report from the European chemical industry.

Several European science societies are also competing for these same funds. The European developmental biologists in the European Developmental Biology Organization (EDBO), the ecologists in the European Environmental Research Organization (EERO), the plant molecular biologists, the cell biologists in the European Cell Biology Organization (ECBO), the neurobiologists and the structual biologists are all separately making attempts to secure funds from the EC and the ESF, for fellowships, training programmes and workshops in their specialist fields. The biochemists have unified their national organizations in the Federation of European Biochemical Societies (FEBS) which, using revenue from FEBS journals, arrange fellowships and workshops. (See table for list of acronyms used in this article.)

The struggle

The power struggle was spotlighted when the EC stopped payments of grant support already contracted for under the SCIENCE programme, which has been making grants for basic research carried out in collaborations. These breaches of contract were precipitated by a commissioner who failed to receive support for his grandiose EC fellowship programme from the European Parliament and the Conference of Science Ministers. Another conflict arose when the German science minister, probably in need of arguments that would convince the national green politicians of the good sense of the genome projects, simultaneously requested reports on human genome research in Europe from the ESF and the Academia Europaea.

These reports presented conflicting views on the future coordination and organization of the programme. The academy suggested an EMBO-like organization, 'Eurogene', for the coordination of the national programmes. ESF, on the other hand, favoured the view that the genome projects should mainly be national enterprises coordinated to avoid unnecessary duplication.

These conflicting views reflect the constitutions of these sources. The Academia Europaea, a European organization consisting of scientists, naturally proposes a pan-European activity, but a foundation deriving most of its support from the national research councils is more inclined to propose networking of national projects. From all the reviews and reports on expanded European projects in biology it is clear that the EC, which is the only organization with its own European funds, favours European projects, whereas the ESF, an organization with funds derived from national sources, tends to defend the national interest.

It is interesting that the majority of those who have been engaged in these numerous reviews are members of the EMBO, and so should have a natural channel for advocating European support through their government representatives on the EMBC, which finances the EMBO. Many of the proposed European projects in biology could be managed by the EMBO if it had a chance to expand. The EMBO members, some 750 of the top biologists in Europe, constitute a coherent group that should be able to work out the

ACRONYMS USED IN THIS ARTICLE

EC	European Commission
ECBO	European Cell Biology Organization
EDBO	European Developmental Biology Organization
EERO	European Environmental Research Organization
EMBC	European Molecular Biology Conference
EMBL	European Molecular Biology Laboratory
EMBO	European Molecular Biology Organization
ESF	European Science Foundation
FEBS	Federation of European Biochemical Societies
HUGO	Human Genome Organization

priorities among themselves and present their proposals more strongly to the EMBC. European biologists are, however, obviously keener to seek support for their specialist area from whatever source looks promising than to remain united and show loyalty to their own organization. It no doubt also suits the EC to offer small sums of money here and there; to divide is to rule.

The problem

Why does Europe have all these competing organizations and why do we lack a body that can harmonize the programmes presented by the EC, ESF, HUGO, EMBL and EMBO, not to mention ECBO, EDBO, EERO and the other European subject-oriented interest groups?

There are several reasons for this competition and the polarization it engenders. Interestingly, most of them lie outside the most obvious cause — the limits on research funds. In most European countries, biological research is well supported even if many research councils have difficulties in executing objective peer review or in taking unpopular decisions resulting from the changes in the quality of research or of national priorities. The genome projects, which rapidly received political support, gave the national research councils in many countries extra funds. One conclusion from the reponse to the Human Genome Project is that scientists in unison can easily convince politicians to provide additional support so long as they present coherent, understandable and attractive proposals. Another factor, of course, is that European politicians, frightened by the US lead in the Human Genome Project, supported European efforts from fear of being left behind.

A second reason for the failure to coordinate European research programmes may be the large group of science administrators who influence decisions at both national and international levels. Most of them have either failed in research or have been trained in law or economics. Even when they have some insight into current scientific problems, their loyalties rest entirely with the organization or the ministry for which they work; they strive to provide identity and importance to their administrative unit rather than to find real solutions to scientific organizational problems. In the rapidly growing field of biological research, where we have difficulty in training sufficient numbers of scientists both for university and industry, this gulf between insufficiently and inappropriately trained administrators and the research community may be greater than in the field of physics, where 30–40 years ago several highly trained physicists opted for administrative rather than scientific careers.

A third contributing factor is certainly that the organization which has by far the largest funds for European collaborative projects, the EC, has a questionable peer review process and often distributes funds on geographical rather than scientific criteria. The limited call mechanism, which restricts applications for support to selected scientists chosen by dubious criteria, is clearly detrimental to a sound research activity.

One reason for the failure to coordinate European Research programmes may be the large group of science administrators who influence decisions at both national and international levels. Most of them have either failed in research or have been trained in law or economics . . . their loyalties rest entirely with the organization or the ministry for which they work . . .

Finally, many representatives of the national science research councils favour the idea that basic science should not be europeanized, but instead, that national efforts should be maintained and strengthened. As well as national pride and the desire to maintain expertise on a national basis, the chief political reason for this policy is that it provides the basis of growth for national industry. But that is not really true. All the relevant industry in this field is multinational, and does not rely on the science effort of single countries. Industry in fact has often more information about relevant partners than the scientists themselves. For instance, the industrial partners Amersham and Bertin have already licensed the multiple oligonucleotide synthesizer developed at the EMBL to be used in the French-British Eureka project on sequence automation.

From the scientific side, increased europeanization offers great advantages. Science can be measured only by international standards. Peer review on a European basis can be performed in a more objective, independent and professional way. (This is especially important for the small countries of Europe.) The skewed distribution of graduate and postdoctoral students can be evened out. It is not feasible for each country in Europe to build up in every field the expertise, which is now the entrance ticket for access to the ever increasing flow of not-yet-published information. Scientific breakthroughs are unpredictable but are fostered by an open research environment containing scientists with different scientific and cultural background, as is clearly demonstrated in the United States.

The remedy

How can we then ease the ever increasing polarization and competition between the different European organizations?

First the scientists themselves must demonstrate that they can set the priorities, that they are prepared to make themselves available for peer review on an international scale and that they can recognize good science within their own subject area but outside their own country. We cannot leave it to the science administrators to achieve the unification because their first loyalty goes to their employer. EMBO has a long record of providing international peer review and given the authority to launch an expansion of European collaboration in several areas of molecular biology it could easily draw on it qualified membership to set up planning committees for such programmes. The plan could then be circulated to all national or European bodies for comments and amendments. This science-based scheme could well become as successful as the current fellowship and course committees of the EMBO and, in the past, the EMBO committee involved in harmonizing the recombinant DNA-technology guidelines. In the 1970s the latter set an example to national legislators of how to separate facts from fiction.

There is also a need for a joint coordinating body for all the various organizations representing both basic and applied areas of biological research in Europe, especially now as we attempt to unify the funding of research beyond the borders of western Europe. It cannot be desirable that organizations representing the research community in basic biological science lack direct contact with the highest levels in the national ministries and as a consequence receive less support than projects in the applied sectors, which are not infrequently inefficiently supervised by bureaucrats and politicians.

A yearly pan-European Conference of Science Ministers should be established to be responsible for coordinating European projects and overviewing and supporting most estabished European organizations representing basic or applied science. Some diversity may be beneficial. The proposal to fuse all European organizations under the EC, as has been suggested, has little to recommend it, because that would place the scientists under the tutelage of unqualified administrators. The EC furthermore suffers from an opaque, bureaucratic, time-consuming, costly and laborious administration that cannot be fountain-head of a European science programme. Proposals from the EC can at present only be vetoed or approved by the Conference of Science Ministers but the latter has no real coordinating power. A pan-European conference of science ministers that can provide the resources directly to the different European organizations based on competing proposals would assure a direct involvement of elected politicians in the harmonization of the European science programmes. Each country may in these deliberations support or reject individual proposals and pay relatively to its gross national product in a similar manner to that successfully applied in the industrial Eureka projects. The administrative costs could be kept at a minimum with this system because it places the management of the funds in the hands of the scientists. □

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Silver Spring monkeys

SIR — As one who attended both State of Maryland vs. Edward Taub trials and served on the search and seizure warrant team that removed documents and animals from Taub's laboratory, I wish to comment on S. B. Galsworthy's letter (*Nature* **349**, 13; 1991).

Galsworthy writes that "Taub was never convicted". Indeed, he was — by a circuit court judge and then, in a later trial *de novo*, by a district court jury. His convictions were for violations of Article 27, Section 59 *et seq.* of the Annotated Code of the State of Maryland, otherwise referred to by law enforcement personnel as "the anti-cruelty statute".

I saw inside Taub's laboratory. The place was abominably filthy, its rusted cages with their broken bars failed to meet federal minimum standards (a matter of public record), all medications had long expired (as shown in the police photographs admitted into evidence), and animals languished with infected wounds (as confirmed in the testimony of primatologist Dr Geza Teleki and four veterinarians with expertise in primate medicine who personally examined the Silver Spring monkeys).

Taub's conduct should be used as an example of how not to do things in the laboratory.

INGRID E. NEWKIRK

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■ Taub's conviction on one count of the original indictment was overturned on appeal and the remaining 118 counts dismissed — Editor, *Nature*.

The parasites

SIR — Efficient scientific work is increasingly dependent on the rapid availability of laboratory reagents. During the past few years, I have as a routine been placing orders by telefax from remote Finland directly to US dealers and obtained the needed items by express delivery within 2–3 days.

Oncogene Science (Manhasset, New York) responded to my recent order for monoclonal antibodies by advising me to contact its new local distributor in Sweden. When I did so, it turned out that the distributor did not keep the antibodies in stock but had to order them as well.

In a second telefax, I made this clear to Oncogene Science, asking it to avoid further delay of a stopover in Sweden by shipping the antibodies directly to Helsinki. In spite of this, the antibodies were delivered through the distributor in Sweden. The consequence was a delay of an additional week and a doubling of the price.

It is reasonable that distributors, who keep local stocks of reagents and provide a rapid service, should charge extra to compensate for their costs. But middlemen who add 100 per cent or more to the price only for changing the address labels on the packages and causing delay are not acceptable. They are just parasites in the scientific community and should be eradicated.

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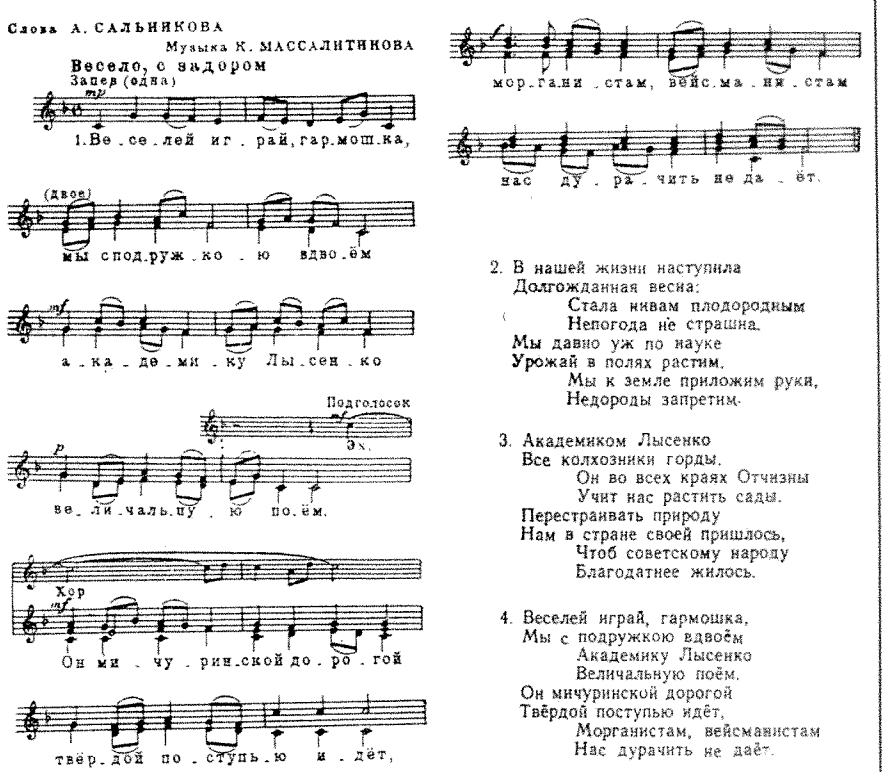
The collective farm song

КОЛХОЗНАЯ ЗАПЕВКА



2. В нашей жизни наступила
Долгожданная весна:
Стала нивам плодородным
Непогода не страшна.
Мы давно уж по науке
Урожай в полях растим.
Мы к земле приложим руки,
Недороды запретим.

3. Академиком Лысенко
Все колхозники горды.
Он во всех краях Отчизны
Учит нас растить сады.
Перестраивать природу
Нам в стране своей пришлось,
Чтоб советскому народу
Благодатнее жилось.

4. Веселей играй, гармошка,
Мы с подружкою вдвоём
Академику Лысенко
Величальную поём.
Он мичуринской дорогой
Твёрдой поступью идёт,
Морганистам, вейсманистам
Нас дурачить не даёт.

1. To the accompaniment of the accordion
My friend and I sing in honour
Of Academician Lysenko*

He follows Michurin's path
With his unerring step
And is not fooled by the followers
of Morgan and Weismann

2. Long-awaited Spring
Has come into our lives
So have fertile fields
That are unafraid of bad weather

Thanks to his teachings
We reap and gather the crop
We have banned bad weather from our fields
By working hard on them

3. Every member of collective farms
Is proud of Academician Lysenko
He teaches the whole country
How to cultivate gardens

And so there is a 'perestroika' in nature
Across the whole country
which will fructify life
of the Soviet nation

4. To the accompaniment of the accordion
My friend and I sing in honour
Of Academician Lysenko

He follows the path of Michurin
With his unerring step
And is not fooled by the followers
Of Morgan and Weismann

THE document reproduced above, a song on the glories of lysenkoism for peasant parties on collective farms, is further evidence to add to that demonstrating the strength of the malign grip exercised by the biologist T. D. Lysenko on Soviet agriculture earlier this century. At the top is the music and the four verses in Russian with a translation by Barbara Izdebska below. The song was unearthed by Dr Yuri F. Bogdanov of the Vavilov Institute in Moscow, and sent on to *Nature* by Professor J. H. Edwards, Genetics Laboratory, Department of Biochemistry, University of Oxford OX1 3QU, UK. Photocopies of the original in full are available from Professor Edwards. Editor, *Nature*

* Lysenko (1898-1976), Soviet biologist, who had a damaging influence on Soviet biology..." (The Macmillan Concise Encyclopaedia).

Dr Baltimore says "sorry"

Dr David Baltimore says he had no knowledge of the fabrication of data in a paper in *Cell* of which he was a co-author, says he will work to develop new guidelines for misconduct and apologizes to Dr Margot O'Toole.

A DRAFT report of the investigation conducted by the Office of Scientific Integrity (OSI) of the US National Institutes of Health into the 1986 *Cell* article on transgenic mice has been issued (see *Nature* **350**, 262; 1991). I welcome OSI's report because of its completeness and detail. I have now had the opportunity to study OSI's findings and to reflect upon the inquiries and proceedings related to both the paper and the data and experimentation that supported the paper's conclusions.

After an exhaustive review of forensic and scientific evidence, OSI has concluded that certain data contained in the notebooks of one of the paper's authors, Dr Thereza Imanishi-Kari, were falsified and/or fabricated, and that, in relying upon such data, Imanishi-Kari presented false and misleading information to the NIH panel charged with investigating the accuracy of the data and interpretations in the paper. I wish to state that if Imanishi-Kari did falsify data or make misrepresentations, I had no knowledge of the misconduct.

The findings do not undermine either the integrity of the work conducted by my postdoctoral fellow, Dr David Weaver, under my supervision or the reliability of our records. However, the OSI was critical of my response to the mounting challenges raised to the work of Imanishi-Kari, my co-author.

The completion of the NIH investigation has prompted me to make these comments, which will address OSI's observations about my conduct and will share the lessons I have drawn from this experience about the appropriate response to such allegations and the respect and candour that must characterize the partnership between the scientific community and the federal government.

OSI criticizes me for my strong defence of Imanishi-Kari, particularly at the May 1989 hearings before the congressional subcommittee, and for my failure to re-examine Imanishi-Kari's data more critically after serious questions had been raised. I wish to state at the outset that my defence of Imanishi-Kari was not due to any lack of regard for Dr Margot O'Toole, the postdoctoral fellow who first uncovered certain discrepancies in Imanishi-Kari's research. I have tremendous respect for O'Toole, personally and as a scientist, and I have consistently maintained that I believe that her analyses were insightful, her expressions of concern were proper and appropriate, and her motives were pure. Rather, my defence of my co-author was fuelled by my respect for Imanishi-Kari's demonstrated abilities as a scientist, by my belief that the paper's scientific conclusions were sound, and by my trust in the efficacy of the peer review process.

The study that gave rise to the paper was conducted as a classic collaboration, with each laboratory performing independent research in its particular area. Mutual respect is the bedrock of any professional collaborative effort, and it was a key ingredient in our particular collaboration, because Imanishi-Kari provided the expertise in serology that I lacked — she possessed proven ability in this field.

O'Toole initially made known her concerns to immunologists at Tufts University. Those experts concluded in June 1986 that there was no evidence of deliberate falsification or misrepresentation and characterized the availability of alternative interpretations of the data as "the stuff of science". A later review at Massachusetts Institute of Technology (MIT) reinforced that conclusion. The expert there found that O'Toole had correctly identified a minor error, but explained that the error was too insignificant to warrant a retraction in the light of "a substantial body of other data that is clear and impressive". The MIT report echoed the sentiments of the Tufts reviewers and noted that "other issues raised by O'Toole, which are largely matters of interpretation and judgement, are best dealt with by allowing the scientific process to take its course." I fully expected that this paper, like all others, would be subjected to the rigours of the scientific peer review process, and that efforts by other laboratories to replicate or extend our findings would ultimately test whether they were correct.

Noeleen Chedd

Baltimore: "temper trust with scepticism".

In January 1989, the first NIH panel to investigate the matter concluded, as Tufts and MIT had, that the science in the paper was essentially sound. The report did, however, raise many issues about the way the data for the paper were produced and, in retrospect, it is evident that I gave too much weight to the overall conclusions of the report and did not appreciate that the report might have had the implication that the results had not been obtained as reported.

In 1989, during hearings before the congressional subcommittee investigating the matter, the Secret Service revealed certain preliminary findings regarding its review of Imanishi-Kari's notebooks. At that time, I continued to base my defence of Imanishi-Kari upon the two university reviews, the January 1989 NIH report and my knowledge of her abilities. I now realize that I erred in failing to heed the warnings and that the better course would have been to suspend further comment on the matter until I had a full opportunity to review and digest all of the new information.

In good conscience I feared a rush to judgement, and I accorded my colleague the benefit of every doubt. I now recognize that I was too willing to accept Imanishi-Kari's explanations, and to excuse discrepancies as mere sloppiness. Further, I did too little to seek an independent verification of her data and conclusions. I acknowledge that, for too long, I focused narrowly on the question of whether the paper could stand; what was important to me was that the solid molecular data gathered by my laboratory seemed to lend credence to the serological findings. In other words, as a scientist, my concern was always for the science: is the result correct? Can it be replicated and built upon?

The OSI report raises very serious questions about the veracity of the serological data. I am shocked and saddened by the revelations of possible alteration and fabrication of data. These discoveries are deeply troubling not only because of their impact upon our article, which has been retraced in the light of these revelations, but because such allegations of fraud undermine public confidence in the entire scientific community. Science must be an objective search for truth. It was my

belief in science and faith in my fellow scientists which led me to set my threshold of suspicion so high.

I wish to state unequivocally that I have never condoned falsity by a scientist. I do not believe it could ever be appropriate to represent that a test that was not performed was in fact completed, or that anything other than the actual results were obtained. Fraud in the laboratory is not only wrong from a moral and legal standpoint, but it impedes the progress of science, as it makes the review and retesting of hypotheses and conclusions impossible. Deliberate falsification demeans all members of the scientific community because it undermines public trust and confidence in our enterprise.

For their work, scientists are entrusted with public funds. I have come better to appreciate the legitimate role of government as the public sponsor of scientific research and to respect its duty to protect the public interest and hold the scientific community accountable for its stewardship of public funds. Such accountability can be entirely consistent with the essential objectivity of scientific inquiry.

The case has highlighted the need to conduct our research and review in a manner the public can appreciate, because continued public support is necessary for the continued life of the scientific enterprise and the nurturing of the academic environment in which we enjoy the freedom to experiment and learn. It is only because public support has been translated into federal financial support that scientists have been able to expand dramatically the range of human knowledge and apply this new knowledge to achieve extraordinary practical advances in such fields as medicine and public health. In the light of this creative partnership, I remain firmly committed to the importance of governmental oversight of federally funded projects, and I look forward to continuing to participate in a healthy and necessary dialogue to improve the process.

I have learned from this experience that the accountability to ensure the responsible use of public funds rests not only with each individual scientist but with the scientific and academic communities as whole. Better self-policing and record keeping will facilitate the government's oversight function and may obviate the need for the repeated hearings and investigations that were needed in this case. This matter has also highlighted the need for clear procedures which guarantee the prompt and thorough investigations of allegations, and I hereby commit myself to participate actively in the study and formulation of new guidelines. Questions raised, whether by junior or senior scientists, must be pursued with vigour, and because junior colleagues may be reticent about alleging outright misconduct, it is incumbent upon those more senior to press for a full airing of their suspicions. Any procedures must include the means to protect those who raise concerns from retribution or discrimination. Scientists must ensure that they do not wait too long or set the threshold too high before calling for the application of close scrutiny to ferret out potential falsity. Finally, the questions raised in this investigation have also underscored the need for greater attention to detail in the handling and recording of data, to further effective peer review and to establish an impeccable record for verification of results.

In conclusion, I commend Dr O'Toole for her courage and her determination, and I regret and apologize to her for my failure to act vigorously enough in my investigation of her doubts. I recognize that I may well have been blinded to the full implications of the mounting evidence by an excess of trust, and I have learned from this experience that one must temper trust with a healthy dose of scepticism. This entire episode has reminded me of the importance of humility in the face of scientific data. **David Baltimore**

The Baltimore case — a chronology

May 1985: Thereza Imanishi-Kari carries out transgenic mouse experiments at the Massachusetts Institute of Technology (MIT) in an attempt to determine if transplanted foreign genes can affect an animal's own genetic material.

April 1986: Original paper based on the mouse data and finding evidence of genetic changes triggered by the transplanted genes is published in *Cell*, with Imanishi-Kari, David Baltimore and others as authors.

May 1986: Laboratory postdoctoral fellow Margot O'Toole discovers an Imanishi-Kari notebook containing 17 pages that suggested to her that some of the key experiments in the *Cell* paper had never been done; at the request of O'Toole, Tufts University, which is preparing to hire Imanishi-Kari, convenes an *ad hoc* committee headed by biologist Henry Wortis to investigate charges.

June 1986: MIT professor Herman Eisen meets O'Toole, Imanishi-Kari and Baltimore to review O'Toole's allegations. His memorandum finds possible minor errors, but no fraud.

October 1986: Contacted by Charles Mapplethorpe, one of O'Toole's former MIT colleagues, National Institutes of Health (NIH) researchers Walter Stewart and Ned Feder begin investigating the case. After they obtain the 17 notebook pages from O'Toole and examine them, they inform NIH officials that they suspect misconduct in the *Cell* paper.

May 1987: NIH Office of Extramural Research begins first inquiry; Tufts committee submits report concluding that there was no deliberate falsification or misrepresentation in the *Cell* paper.

September 1987: After a year of NIH review, Stewart and Feder receive permission to try to publish their 34-page critical analysis of the *Cell* paper, in which they conclude that Imanishi-Kari's experimental records contradict some of the paper's key conclusions. Over the next year *Cell*, *Science* and *Nature* all reject the paper. It is never published.

May 1988: Congressional Investigations subcommittee of Representative John Dingell holds its first hearings, focusing on the response of Tufts and MIT to the O'Toole allegations; Baltimore issues a "Dear Colleague" letter attacking Dingell and asserting that congressional interference "is totally unnecessary".

July 1988: Dingell subpoenas Imanishi-Kari's laboratory records and turns the notebooks over to Secret Service for analysis.

November 1988: Baltimore and Imanishi-Kari publish a correction in *Cell* indicating that the original paper contained an 'overstatement' of the specificity of BET-1, a key reagent.

January 1989: First NIH investigation ends, finding "significant errors of misstatement and omission . . . but no evidence of fraud, conscious misrepresentation, or manipulations of data." In a letter, NIH director James Wyngaarden chastises the *Cell* paper authors: "Even though the allegations have been known to you . . . at least since spring of 1986 . . . you never met to reexamine the data." Such a meeting, he writes, "may have made a full investigation unnecessary."

April 1989: Based on new evidence from the continuing Dingell investigation and subsequent O'Toole findings, NIH reopens the investigation within the newly created Office of Scientific Integrity (OSI).

May 1989: Dingell holds two hearings, at the first of which the Secret Service testify that 20 per cent of a critical notebook is forensically questionable; at the direction of NIH, Baltimore and Imanishi-Kari publish a second correction in *Cell*, giving additional data on the specificity of BET-1.

Summer 1989: Baltimore publishes an article in *Issues in Science and Technology* giving his side of the story and attacking Stewart, Feder and the Dingell staff for unwarranted meddling. "If the sad history of this investigation demonstrates nothing else, it shows that uninformed or malinformed outsiders cannot effectively review the progress of scientific activity," he writes.

May 1990: Dingell holds fourth hearing. Secret Service investigators present additional forensic data showing that Imanishi-Kari's notebook records and purported experiments were "not contemporaneous with respect to time." Findings cast doubt on the data in the second *Cell* correction.

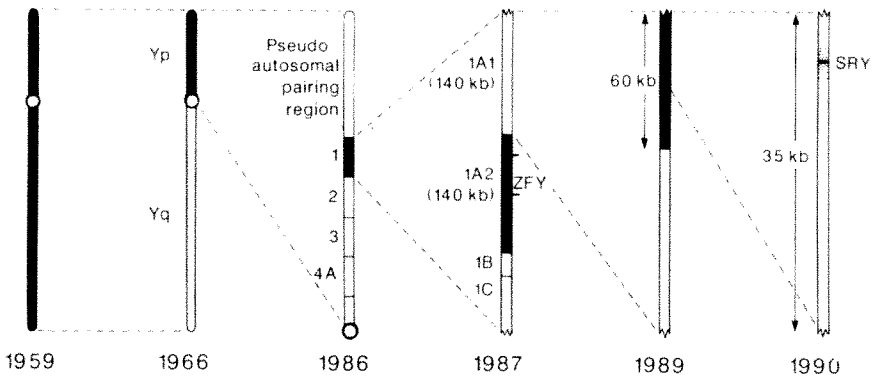
March 1991: Draft report of second NIH investigation reverses previous report, finds "serious scientific misconduct", including data fabrication. Dingell staff announce plans to hold hearings in May on "who-knew-what-when." OSI also states intention to pursue allegations of a cover-up. Baltimore announces that he will retract the *Cell* paper. **C.A.**

SEX DETERMINATION

The making of male mice

Anne McLaren

LAST year saw the discovery[1,2] of the best candidate yet for the long-sought-after testis-determining gene in mammals (see figure). Its claims rested initially on its localization: in both the human and the mouse, it was located within the region that constituted the smallest amount of Y chromosome DNA known to induce masculinization, and in the human it appeared to be the only gene within the relevant 35-kilobase region. It was therefore named *SRY* (human), *Sry* (mouse), for sex-determining region of the Y chromosome.



The hunt for the testis-determining factor from 1959, when the Y chromosome was shown to be male-determining in both mouse and man, to 1990 and the identification of the sex-determining region (*SRY* in humans, *Sry* in mice). For references see the previous News and Views article on the subject[9].

The candidacy of *Sry* was strengthened by the observation that it was expressed in the developing mouse gonad at the expected time for testis determination; and unlike the previous best bet, *ZFY*, it was not expressed in the developing ovary of mouse embryos in which the testis-determining gene on the Y chromosome (*Tdy*) was known to be defective. Within a few months, further support was forthcoming: *Sry* turned out to be expressed in the gonadal somatic cells previously shown to be responsible for testis determination[3], the gene was absent from the *Tdy*-defective mouse mutant[4], and two sex-reversed XY women were found to have mutations in *SRY* that were not present in their father's gene[5,6]. It seems that an intact copy of *SRY*/*Sry* is necessary for testis determination. But is it, in the absence of the rest of the Y chromosome, sufficient?

For mice, the answer is yes, at least sometimes. On page 117 of this issue[7], Koopman *et al.* describe 11 XX mice transgenic for *Sry* on a 14-kilobase DNA fragment. Three were sex-reversed males, eight were females.

The point is made. *Sry* alone can induce maleness; but certain unsatisfactory features remain. Why only three sex-reversed males? The transgene was inserted in multiple copies: was this a factor? Did the three males carry more copies of *Sry* than the eight females? We are not told. Was *Sry* expressed in all 11 transgenics, or did the eight females fail to express it because it was inserted in an inappropriate region of the genome? One of the transgenic females has transmitted the transgene to her progeny, which should allow expression to be examined in the embryonic gonad, but this crucial information is not yet available.

Koopman *et al.* have also produced mice transgenic for the human *SRY* gene. These raise yet more questions. Three integrations were achieved, giving rise to two transgenic lines, and transgene expression in the developing gonads was demonstrated, but no sex-reversal of XX embryos was seen. Would sex reversal have been achieved if more *SRY* transgenics had been made? Or is the human *SRY* product ineffective in the mouse? Or perhaps *Sry* is sufficient to determine maleness, but *SRY* alone is not? Of the four XX human individuals who showed masculinization with only 35 kilobases of *SRY*-containing Y chromosome DNA, all developed testicular tissue but none showed fully normal development of male genitalia[8].

Most of these questions will probably be answered in the next few months. If mammals turn out to resemble *Drosophila* and *Caenorhabditis* in having a complex cascade of sex-determining genes, the sequence will take longer to unravel. We know that *Sry* must be part of that cascade; what the transgenic results tell us is that the other genes in the cascade, whether they precede or follow *Sry* in the regulatory sequence, must be on the autosomes or on the X chromosome, not on the Y. It seems that *Sry* is indeed *Tdy*, the testis-determining gene on the Y. □

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4. Gubbay, J. *et al. Development* **109**, 647–653 (1990).
5. Berta, P. *et al. Nature* **348**, 448–450 (1990).
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7. Koopman, P., Gubbay, J., Vivian, N., Goodfellow, P. & Lovell-Badge, R. *Nature* **351**, 117–121 (1991).
8. Palmer, M. S. *et al. Nature* **342**, 937–939 (1990).
9. McLaren, A. *Nature* **346**, 216–217 (1990).

COSMOLOGY

Dark dark matter

Craig J. Hogan

AMONG the suggestions that the Universe is made mainly of invisible material, one of the most predictive is a proposal put forward by Sciama[1,2]. In this scheme, this 'dark' matter includes neutrinos with a mass of 27.7 ± 0.5 electronvolts (about 0.005 per cent of the electron's mass). The particles are not however entirely dark: they decay occasionally (the lifetime being of the order of 10^{23} seconds) to produce ionizing photons with an energy of 13.8 ± 0.2 electronvolts sufficiently copiously to ionize gas in galaxies today and in the distant intergalactic medium. Until recently these hypothetical decays have eluded direct observational constraints. But the hypothesis has now been dealt a blow by two similar experiments[3,4], one reported on page 128 of this issue[3], which show that the mass of dark stuff in galaxy clusters does not have the ultraviolet glow which would have been expected if it included such decaying neutrinos.

Massive neutrinos have long been a favourite hypothetical candidate for the dark matter that pervades the Universe. The total number of neutrinos can be predicted from the way they would have interacted with the hot radiation early in the Universe. So we know that if the one dominant species had a mass of about $100h^2$ electronvolts, where h is the present rate of expansion of the Universe (Hubble's constant, in units of 100 km s^{-1} Mpc^{-1}), it would just suffice to give the Universe the magical critical density, long preferred on aesthetic grounds, which makes space 'flat' (devoid of gravitational curvature) and gives the Universe zero total energy.

A new twist was given to this idea by Sciama, who noticed that if such particles decayed slowly, it would help to solve several other cosmological puzzles by providing a diffuse source of ionizing photons to strip electrons off atoms in intergalactic and interstellar gas. In the interstellar medium, we have an accurate calibration of the electron density along several lines of sight to pulsars, and very simple models of ionization by stars fail to predict this sufficiently uniformly. The distant intergalactic medium is known to be highly ionized, from the absorption spectra of distant quasars, and the source of this ionization has not yet been identified. Sciama showed that both puzzles could be explained

if cosmic neutrinos decay into ultraviolet photons. He used various arguments to limit severely the range of allowed masses and lifetimes of these decaying particles, thereby making a highly vulnerable, falsifiable theory.

Although highly speculative, interest in the idea mounted over the past year as evidence has appeared from several unrelated directions that at least some neutrinos have mass and decay channels (see Nelson's News and Views article[5]). And the most elegant accepted explanation of the solar neutrino deficit, especially for low-energy neutrinos, involves a conversion between massive electron and muon neutrinos catalysed by the solar plasma. The preferred picture predicts that the more massive of the two has a mass of about 0.001 electronvolts. Laboratory experiments have recently suggested[5] that a very massive neutrino exists with a small mixing (about 1 per cent) and a mass of about 17,000 electronvolts. If real, this neutrino would need to decay very early in the history of the Universe to avoid dominating its mass and energy budget by a large factor. As there are three neutrino species to play with (under some restrictions, possibly more), and the origin of their masses is poorly understood, the relationship between these results is still obscure, but for just this reason they have served to highlight the central importance of neutrino masses as a way to probe new physics.

Sciama's idea offers another astrophysical window on neutrino properties, as it suggests that neutrinos slowly convert to observable ultraviolet light. Clusters of galaxies represent large concentrations of dark matter — indeed, their dynamics provide some of the strongest evidence that dark matter exists — so careful observations should reveal the photon glow from their steady radioactive decay, if it is as rapid as Sciama requires for it to ionize gas. Until now, it has been difficult to look for this glow because the light has appeared in the ionizing ultraviolet region of the spectrum at which the pervasive cosmic hydrogen is extremely opaque — so opaque that we cannot even see out of a local region of our own Galaxy. The solution to this part of the problem is to look at distant, rapidly receding clusters; the predicted decay line radiation lies at a wavelength only just above the ionizing threshold, so that the small redshift induced by the recession suffices to bring it below the transition to wavelengths at which interstellar gas is transparent. Although the Earth's atmosphere is still highly opaque in this part of the ultraviolet, with spacecraft it is possible to make the observation.

Davidsen *et al.* who report in this issue[3], used the Hopkins Ultraviolet Telescope on the recent shuttle-borne Astro-1 mission, while Fabian *et al.* used the International Ultraviolet Explorer satellite. Both groups examined selected galaxy clusters (at redshift 0.18 and 0.646 respectively) and found no evidence of spatially extended line radiation at a level up to 30 times fainter than the simplest models predict.

As always, there are caveats: for example, none of the clusters have precisely determined masses, which leads to uncertainties in the predicted surface brightness even if the neutrino properties are exactly known. Even so, it does not seem possible to make the clusters faint enough to agree with the data. Although small amounts of absorbing neutral gas in the local vicinity of the cluster can absorb the light before it gets redshifted below the ionization threshold, it seems unlikely that the neutral gas would so completely cover the cluster that it would totally obscure the light: to survive in the high-temperature cluster environment, cold gas would need to be highly clumped in clouds. Even if the direct line radiation is completely absorbed, the absorbed light eventually gets out as fluorescent line radiation from the atoms, so careful observations should be able to close the remaining loopholes. At present, it seems unlikely that dark matter decay can provide enough photons appreciably to alter the ionization balance in the interstellar medium. □

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POPULATION GENETICS

Farming is in the blood

J. S. Jones

When people move, they take their genes with them. On page 143 of this issue[1], Sokal *et al.* show that patterns of inherited variation in modern European populations still reflect the migrations of ancient farmers who spread from the Middle East. Ten thousand years ago, genetic change was driven by economic advance. Remnants of the same process can be seen all over the world today.

Mary Evans

Early farmers in Europe — spreading the fruit of their labour.

Farming started in Mesopotamia about 9,000 years ago, and covered the whole of Europe within four millennia. Its spread can be followed by the appearance in the archaeological record of artefacts such as decorated beakers. There are two views of the spread of culture — the diffusionist, which emphasizes the passage of new techniques by learning, and the migrationist, which sees mass migrations as the key to ancient economic, social and political change. Genetic patterns suggest that an intermediate process was involved in Neolithic Europe. Economic advance led to population growth of the farmers and hence to their geographical expansion into and intermarriage with the native population. The hunter-gatherers of Mesolithic Europe suffered a process of gentrification — or even yuppification — from the east, and it is easy to imagine the complaints of the archers (or stone-throwers) as the incomers with their new-fangled ways, high-technology imports and braying voices moved in on what had been a rural idyll.

Whatever the cultural clashes between old and new Europeans, they were not enough to act as barriers to mating. The survey of Sokal *et al.*[1], of 26 polymorphic systems, including blood groups, enzyme variants and histocompatibility antigens, in over 3,000 places in modern Europe, shows that there is an overall trend in genetic similarity from south-east to north-west (although the ABO blood groups deviate from the general pattern). A map of the spread of agriculture built up from the dates of the latest Mesolithic or earliest Neolithic settlements in each region looks remarkably similar to that of genetic distances. There is a strong correlation (which is independent of simple geographical position) between the genetic structure of modern populations and the local dates of origin of agriculture.

All this suggests that what Sokal *et al.* call a process of demic diffusion was involved as the wave of advance of farmers spread at about one kilometre a year. As new farms were founded at the edge of the expanding population, the agriculturalists absorbed the genes of the natives. This process began in the Balkans, and was completed thousands of years later on the western fringes of Europe, producing the genetic trends seen

today. Only one recalcitrant tribe — the Basques — resisted the blandishments of the new technology, and as a group they remain genetically distinct from all other Europeans[2].

Economic change has often led to human movements whose genetic effects can persist for long periods. The ancestors of Black Americans came to their new home against their will. They brought many genes in addition to those controlling skin colour. Nearly all carried a variant allele at the Duffy blood group locus which is native to west Africa (where it confers protection against malaria) and is absent from whites. But up to a quarter of Duffy alleles in modern American Blacks are of the European type, showing the extent of intermarriage between the two populations over the past 300 years[3]. Such economic relics persist even in Britain. In 1108, King Henry I moved a group of artisans from the banks of the Tweed to Pembrokeshire, where they set up a weaving industry. Eight hundred years later, their descendants retain a characteristic set of blood group frequencies which differentiates them from their Welsh neighbours[4].

In King Henry's day, as in modern times, those who could afford it preferred silk to wool. The Silk Road is one of the most ancient of all trade routes and for 2,000 years linked the old Chinese capital of Changan to the Mediterranean. A new survey of haemoglobin variants in a quarter of a million Chinese[5] shows some clear trends along the Road. There are many abnormal alleles at its western end, suggesting that Mediterranean traders brought with them far more than bills of exchange. Five hundred years ago a shift northwards in the position of the Silk Road from desert into grassland allowed horsemen from the first time to travel fast enough to bring plague into Europe from China. Differential susceptibility to plague by individuals of different blood group may explain why the ABO blood group cline in Europe does not follow the spread of agriculture. This is speculation, but even if it is wrong it emphasizes how much any genetic reconstruction of the past depends on the assumption used: a little bit of natural selection can soon erase the effects of history.

Economic development has led to a dramatic increase in migration over the past century. Anthropologists of the future will face a much more complex pattern of human movement than that which exists at present. Fortunately, it seems certain that today's migrants will be just as punctilious in leaving their genetical visiting cards as were our own ancestors. □

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GRAVITATIONAL WAVES

Recycling for a cleaner signal

Frederick J. Raab

OBSERVATION of gravitational waves, the ripples in spacetime emitted by violent cosmic events such as the deaths of stars and the births of black holes, could revolutionize our understanding of astrophysics. Several groups are engaged in attempts to build gravitational-wave observatories based on advanced laser interferometers. Efforts at this frontier have spawned new techniques, such as light recycling and the use of squeezed light to maximize the detectors' sensitivity. A new tool, called dual recycling, has been demonstrated[1] which allows more efficient use of laser light and provides an elegant method of tailoring the bandwidths of these detectors.

A passing gravitational wave causes distances between inertial masses to change by some fractional amount. This effect is measured in an interferometer (see box) by bouncing light beams split from a common laser between mirror-coated test masses in each of two perpendicular interferometer arms, then recombining, or interfering, the beams as they emerge from the arms. If the distances between the masses in both arms are exactly equal, the light recombining at the beam splitter returns towards the laser, leaving a strategically placed photodetector in darkness. The absence of light at this photodetector is referred to as a dark fringe, in contrast to the bright fringe returned to the laser by the beam splitter.

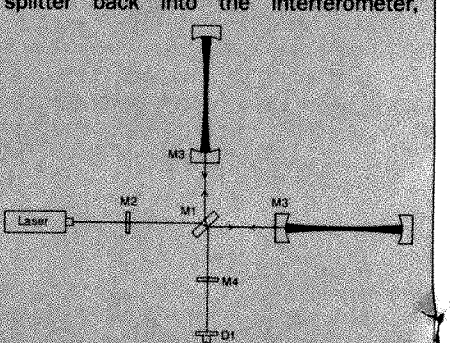
A gravitational wave incident on the detector would upset the balance of the arms' lengths thereby switching some light from the bright fringe onto the photodetector. Because gravitational waves interact weakly with matter, the rich information they carry is preserved as they propagate through space but their influence on detectors is feeble. Predicted signals for the gravitational-wave observatories correspond to fractional displacements of $h = \Delta L/L \approx 10^{-21}$, where ΔL is the difference between the two arm lengths, and L is their average length. Test masses separated by several kilometres will move much less than a nuclear diameter. Laser-interferometer prototypes with a resolution of a few per cent of a nuclear diameter over tens of metres operate today, but further progress is needed.

Once the test masses are sufficiently isolated from disturbances attributable to seismic noise and other random forces, the ability to detect a change in the dark fringe fixes the detector's sensitivity. In the 'shot-noise' limit, fundamental fluctuations in photon number inherent in the quantum nature of light make the smallest detectable signal from the interferometer inversely proportional to the square root of the power in the bright fringe.

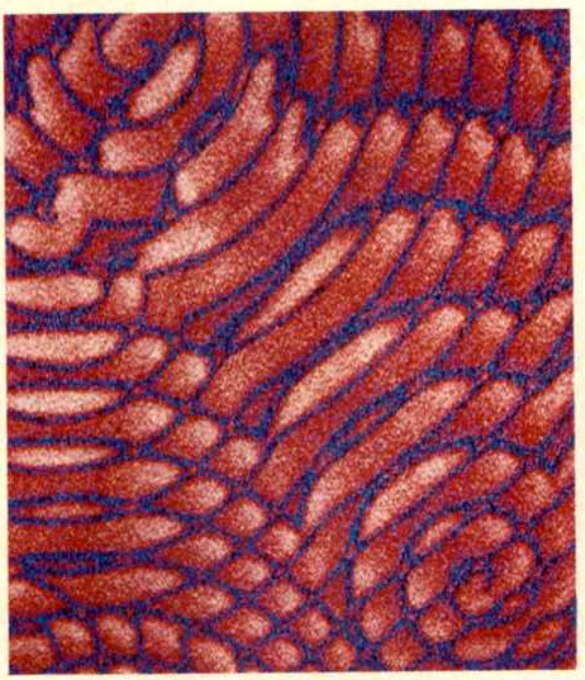
Light recyling[2] increases the bright-fringe power obtained with a given laser, in principle achieving the equivalent of a thousand-fold increase in laser power: in broadband recycling (operating over a wide range of gravitational wavelengths) a mirror inserted between the laser and the beam splitter reflects the bright-fringe light back into the interferometer, recycling the photons for another measurement. An alternative technique, resonant recycling, uses a combination of mirrors to switch photons between the two arms in synchrony with the oscillations of a given gravitational wave, allowing the signal to build up over many cycles. In addition to increasing the bright-fringe power, this technique converts the interferometer into a more sensitive, tuned instrument for detecting periodic signals. Dual recycling[3] involves recycling both the bright fringe and any signal that would appear at the photodiode, allowing it to build in strength. Although it is conceptually similar to resonant

Laser interferometer with dual recycling

Laser light illuminates the beam splitter (M1) after passing through the power recyling mirror (M2). The beam splitter directs light into two perpendicular interferometer arms where the light is stored between an input mirror (M3 or M3′) and an end mirror (unlabelled), bouncing many times between these mirrors (denoted by thick lines). In the delay-line configuration, this light illuminates different spots on these mirrors on each pass; in the Fabry–Perot configuration the same spots are illuminated on each pass, thereby forming an optically resonant cavity with these mirrors. Light returning from the arms interferes at the beam splitter. If both beams have travelled exactly the same distance, all of the light is returned toward the laser; any slight imbalance in the arm lengths causes some light to propagate towards the photodetector (D1). This light is the interferometer output signal. The power recycling mirror reflects light returned by the beam splitter back into the interferometer, thereby increasing the circulating power. The signal-recycling mirror (M4) allows the signal to build up in the interferometer in a similar manner before detection at D1.



Wave upon chemical wave



As indicated in Ian Stewart's recent News and Views article (*Nature* **350**, 557; 1991), coupled oscillators can offer some spectacular sights. The geometric complexity resulting from two coupled chemical-wave systems, pictured on the right, attests further to the fact. The spiral waves are formed on a polymer (Nafion) membrane loaded with ferroin and immersed in a mixture of the Belousov–Zhabotinsky reagents. The ferroin catalyses this oscillatory reaction, generating wavefronts that propagate on both sides of the membrane. The two sets of waves can communicate by transport of one reagent through the membrane. On page 132, K. Showalter *et al.* show that this interaction can lead to behaviour ranging from irregular patterns to entrainment and phase locking. It is tempting to infer that this kind of phase-sensitive communication could occur across cell membranes. The wavefronts are seen here in blue on both sides of the translucent membrane.

recycling, dual recycling can operate over a wide range of bandwidths.

The new work by K. A. Strain and B. J. Meers[1] demonstrates that dual recycling can improve the broadband low-frequency performance of a particular interferometer configuration which uses optical delay lines to store light in its arms. With this improvement the delay-line interferometer should be as sensitive as a Fabry–Perot interferometer. (Practical constraints on the size of mirrors had previously favoured the more compact Fabry–Perot configuration for detecting gravitational waves at frequencies below a few hundred hertz because the maximum storage time for light in a delay line was too short.) For their demonstration, Strain and Meers built a small-scale broadband recycling interferometer in which the light made a single bounce in each arm. Because the mirrors were not suspended as in a real gravitational-wave detector but were held in rigid mounts, vibrations dominated the displacement noise below 20 kHz. Above this frequency, the displacement noise was shot-noise limited at a level determined by the available bright-fringe power and the very short storage time of light in the arms.

When the signal recycling mirror was added, a sevenfold increase in signal-to-noise ratio was observed. This increase was slightly better than the expected sixfold improvement, a fact which Strain and Meers attribute to the improved quality of the optical wavefront inside the interferometer owing to dual recycling.

Dual recycling also looks promising to those of us who favour the Fabry–Perot interferometer design. Dual recycling can adapt a broadband recycled interferometer to narrowband operation by adding a single mirror to the output side of the instrument. Adjusting the position of the signal recycling mirror tunes the interferometer to a particular frequency, which might be chosen to correlate with the rotation frequency of a known pulsar. The detector's bandwidth can be varied by adjusting the transmission of the signal recycling mirror. In some cases an intermediate bandwidth may be desirable, trading off some bandwidth for improvements in mode quality within the interferometer.

A very different method for improving interferometer sensitivity in the shot-noise limit is to inject 'squeezed' light into the interferometer at the beam splitter[4]. The presence of squeezed light can alter the stochastic nature of the beam splitting process, reducing quantum fluctuations in the output beam at the cost of increased fluctuations in radiation pressure on the test masses. Although squeezing cannot violate the uncertainty principle, it may allow the quantum limit to be reached with less bright-fringe power. The improvement in precision has been confirmed in a simple interferometer[5] and the evaluation of squeezing techniques in practical gravitational-wave detectors has begun[6]. For squeezed light to be effective, interferometer losses must be minimized, a situation which also enhances recycling efficiency.

Where will this lead? Gravitational-waves

RÉSUMÉ

Fit connection

Two years after the mapping of the gene which causes a rare form of neonatal epilepsy, a Finnish group reports the localization of the gene for an adult form of the disorder (A.-E. Lehesjoki *et al. Proc. natn. Acad. Sci. U.S.A.* **88**, 3696–3699; 1991). Progressive myoclonus epilepsy of Unverricht–Lindberg type was first described 100 years ago, and is especially common in the Baltic region. Performing linkage analysis with 12 Finnish families, Lehesjoki and colleagues excluded 25 per cent of the genome before tracking down the disease locus to position 22.3 on the long arm of chromosome 21. The most promising candidate gene which maps to the same region encodes the β subunit of the calcium-binding protein S100, expressed mainly in glial cells.

Golden opportunity

The discovery in 1984 that certain phospholipids spontaneously arrange themselves into microtubular structures led C. R. Martin and colleagues to see if the use of microporous materials as templates would extend the range of microtubular materials. To the surprise of Martin and C. J. Brumlik (*J. Am. chem. Soc.* **113**, 3174–3175; 1991) these structures are not restricted to organic molecules. Gold can be persuaded to coat the pores of microporous alumina, without blocking them. The alumina can then be etched away to leave tubules with a diameter as little as 0.1 μm and a length of up to 2 μm. One possible application, says Martin, would be to use plugs of gold microtubules, which have a large surface-to-volume ratio, in flowing electrochemical reactors to reduce solutions.

RNA action

Many of the processes going on inside living cells are carried out by complex machines made up of both proteins and RNA – perhaps the best known case is the translation of messenger RNA by ribosomes, but other more recently fashionable examples include splicing of RNA and protein export. Inspired by these precedents, L. Young *et al.* (*Science* **252**, 542–546; 1991) took another look at the event that creates it all in the first place, transcription of DNA. In eukaryotes, transcription requires, in addition to RNA polymerase itself, a whole array of auxiliary factors, all of which were assumed to be proteins. Not so, it seems. Young and colleagues have found a factor that is essential for transcription by RNA polymerase III in silkworms and that consists, at least in part, of RNA. The number of biological processes that do not require this material shrinks further.

from sources that can be predicted with confidence, such as coalescing neutron star binaries, should be detectable with long-baseline interferometers using modest broadband recycling factors (about 100) and laser powers (about 60 watts) that should become available in the near future, even without squeezing. The use of squeezed light with broadband recycling configurations should either relax demands for bright fringe power or further extend detector sensitivity. The relative weighting of these techniques in future detectors will hinge on developments in optics, lasers and materials — fields that are now progressing rapidly. As we look to a new frontier in astronomy we should remember a lesson learned from the opening of a previous frontier, radioastronomy. The Universe was far richer in sources than could previously have been imagined and nobody ever complained that their instruments were too sensitive. □

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VIROLOGY

Architecture with a difference

David J. Filman and James M. Hogle

With the description by Rossmann and colleagues of the structure of canine parvovirus at 3.25 Å resolution[1], we have the first example of the architecture of a virus with a single-stranded DNA genome. Although the protein coat (capsid) of canine parvovirus includes a core structural motif that has frequently been observed in RNA viruses, the arrangement of the motif differs considerably from anything known previously. The new structure provides several insights into the assembly and biological properties of simple viruses.

The architecture of small viruses is dictated by the need for the 'blueprint' for a large and elaborate protein coat to be encoded by a relatively small genome. This requirement is satisfied by constructing the coat from several copies of small protein subunits which pack together in a regular arrangement to form a particle either with helical symmetry (for filamentous viruses) or icosahedral symmetry (for 'spherical' viruses). An icosahedrally symmetrical capsid is constructed from 60 copies of some repeating unit, with one or more proteins in each unit. In most icosahedral viruses the capsid proteins contain a wedge-shaped core motif (consisting of an eight-stranded antiparallel β barrel), with each motif representing protein of relative molecular mass about 20,000 (M_r 20K) (Fig. 1).

Indeed, among the published structures of small icosahedral viruses the only exception to this generalization is MS2, a small RNA bacteriophage, whose coat proteins form dimers that are remarkably similar to the α1 and α2 domains of the class I major histocompatibility antigen[2]. But a shell constructed of 60 copies of a minimal core motif is large enough only to encapsidate a genome encoding little more than the coat protein itself. This is the case with satellite tobacco necrosis virus which depends on a helper virus to supply other proteins necessary for replication[3]. Viruses which code for their own replicative enzymes build larger shells either by forming the repeating unit from multiple copies of a single subunit (for example 180 copies of one kind of subunit in the 'T=3' plant viruses[4,5]), by forming the repeating unit from several chemically distinct wedge-shaped protein domains (for example the picornaviruses[6,7] and comoviruses[8]), or by using a single, much larger, protein subunit.

The canine parvovirus (CPV) particle has an outer diameter of 250 Å, and so is intermediate in size between satellite tobacco necrosis virus (180 Å) and the T=3 plant viruses and picornaviruses (300 Å). Its 5,000-base DNA genome is roughly the same size as the RNA genomes of the T=3 plant viruses, and encodes the capsid proteins and two nonstructural proteins which function as transcription regulators. Early studies showed that the virion, the infectious particle, is composed of variable numbers of three large proteins, VP1 (M_r about 85K), and VP2 and VP3 (each of M_r about 65K). These observations were difficult to reconcile with the need for an icosahedral capsid to contain a multiple of 60 subunits, until sequence analyses revealed that VP1 is derived by an alternative splice of the capsid message (adding an additional 20K domain to the N terminus of VP2), and that VP3 is derived by post-translational cleavage of 15–20 residues from the N terminus of VP2. Thus, the virion contains exactly 60 copies of the common minimal 'shell-forming' sequence of VP3. Remarkably, however, this minimal sequence is more than twice as large as the capsid-forming subunits observed in the other virus structures, though it contains only one wedge-shaped core motif. The unusually large size of the protein (and hence the ability to form a 250 Å shell from only 60 copies of a single protein) results from the presence of several large loops connecting the strands of the β-barrel core, including an especially large loop (231 amino acids) connecting the G and H strands (Fig. 1).

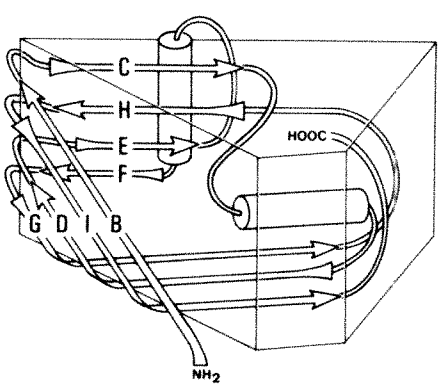

FIG. 1 Schematic representation of the wedge-shaped eight-stranded antiparallel β barrel core motif shared by the major capsid proteins of several icosahedrally symmetrical viruses. The proteins which contain this common folding pattern have structurally similar cores, but exhibit great diversity in the loops which connect the β strands, and in their N and C extensions. Individual β strands are shown as arrows, and are labelled alphabetically. The flanking helices, which are indicated by cylinders, are not absolutely conserved, but are common to many virus structures, including that of canine parvovirus.

The arrangement of capsid protein subunits in the shell of CPV is not unexpected, but it represents a previously unseen mode of icosahedral packing. Although an infinite variety of choices is available for selecting the shape of each of the 60 repeating units which tessellate an icosahedral surface, among the simplest choices are the 'triangular' wedge bounded by a fivefold and two threefold axes of symmetry, and the quadrilateral 'kite-shaped' wedge bounded by a fivefold, threefold and two twofold axes of symmetry (Fig. 2, left). In the known icosahedral virus structures, the subunits are fairly compact and, to a good approximation, the 'geographical' limits of the protein subunits happen to coincide fairly well with the boundaries of either the triangular or the kite-shaped choices of icosahedral unique volume. In the T=3 icosahedral plant viruses, each triangular area contains three chemically identical copies of the capsid protein (labelled A, B and C in Fig. 2); in the picornaviruses, the biologically relevant protomer includes three structurally similar but chemically distinct copies of the core motif (labelled VP1, VP2 and VP3), arranged to occupy approximately the kite-shaped area. Satellite tobacco necrosis virus is the one-domain analogue of the T=3 viruses, with each capsid protein occupying the triangular asymmetric unit (Fig. 2, top right). Surprisingly, in CPV the capsid protein occupies the kite-shaped asymmetric unit (Fig. 2, bottom right), and thus it can be thought of as the first example of a single-domain analogue of the picornavirus architecture.

The CPV structure includes several other unusual features. One is the presence of strong electron density corresponding to at least 11 nucleotides of icosahedrally ordered DNA (per protein subunit) bound to the

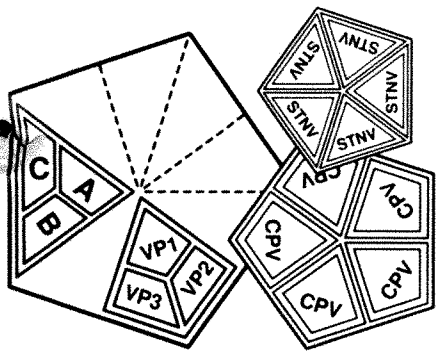

FIG. 2 The architectures of small icosahedrally symmetrical viruses illustrate some of the ways to form a closed protein shell from 60 identical copies of a simple shape. The 'triangular' choice of repeating unit is occupied by one capsid protein subunit in satellite tobacco necrosis virus (STNV), and by three chemically identical subunits (A, B and C) in the T=3 icosahedral plant viruses. The 'kite-shaped' repeating unit is occupied by a single large subunit in canine parvovirus (CPV), and by a protomer that includes three chemically distinct large subunits (VP1, VP2 and VP3) in the picornaviruses. Five copies of either simple shape can form a pentagon (as shown); and 12 pentagons can be arranged to form a closed surface.

inner surface of the capsid. Similarly well-ordered nucleic acid structure has been observed only once before, in bean pod mottle virus[8]. Being able to see a protein–nucleic acid complex is valuable to our understanding of the recognition process, both because there are few crystalline examples of biologically relevant complexes, and because the recognition of virus-specific nucleic acid is an important mechanism in the control of viral self-assembly.

A second striking feature is the presence of a hollow tube at each fivefold axis, extending from the inside of the particle to the outside. The tube, which is at least 8 Å in diameter throughout its length, is formed by the association of five copies of a two-stranded β sheet contributed by residues in the loop connecting the D and E strands at the narrow end of the β-barrel core. The presence of strong electron density on the inside of this tube in virions, but not in naturally occurring 'empty' particles, the observation that the N-terminal domain of VP1 is susceptible to proteolysis in virions but not in empty capsids, and the presence of a well-conserved sequence of small amino acids in the polypeptide strand which connects the N-terminal domain of VP1 to the β-barrel core, all suggest that the cylindrical structure furnishes a route for the externalization of up to 12 copies of this domain when a full complement of viral DNA is present.

Finally, the new structure provides a framework for investigating the determinants of host range and antigenicity in canine parvovirus. Because only four changes in the capsid protein sequence (and three silent mutations) are sufficient to permit the related feline panleukopenia virus to grow in dogs, CPV is an appropriate model system for investigating the mechanisms of host-range restriction. In addition, because there is no evidence for its existence as a pathogen earlier than 15 years ago (it is now endemic among wild and domestic dogs), CPV is an excellent system for addressing the related question of how viruses nominally specific for one host become adapted to infect another. Given the recent identification of a variety of novel pathogens in humans, this question is of more than casual interest. □

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BLACK HOLES

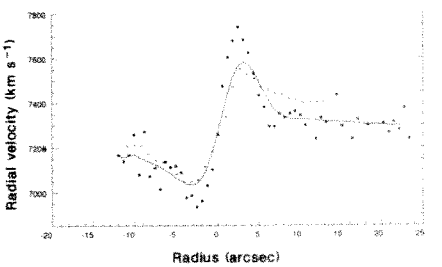
Bigger and better

James Binney

A BLACK hole more massive than any so far identified seems to lie at the heart of the distant galaxy NGC6240 — or so suggest Bland-Hawthorne *et al.* in the 10 April issue of the *Astrophysical Journal*[1], on the basis of high-resolution studies of the dynamics of gas in the galaxy.

Astronomers have long believed that black holes with masses as large as 10^9 solar masses ($M_\odot$) lurk at the centres of many large galaxies, because in the conventional model of a quasar — an object no bigger than the Solar System yet often bright enough to be seen right across the Universe — the system is powered by the accretion of gas onto a massive black hole. Actually finding one of these black holes has proved extremely difficult, however. A black hole can be detected only through its gravitational field, which in effect means by studying the motion of gas and stars near it. At a separation of about 50 parsecs (150 light-years), the speed of a star on a circular orbit around a 10^9 $M_\odot$ black hole becomes comparable to the typical speed, about 300 km s^{-1}, of a star at the centre of a giant galaxy. So to detect a 10^9 $M_\odot$ black hole by its effect on the motion of stars in the nucleus of a galaxy, one has to be able to resolve structure within the galaxy's central 100 parsec. For the nearest appropriate galaxies, numbers 31 and 32 in Messier's catalogue, this distance translates to 36 seconds of arc, so measurements of the required resolution are readily obtained from the ground in the optical band. But by Murphy's law these nearest galaxies do not contain super-massive black holes, and the evidence that they harbour less massive black holes is still not firm (though it is fairly convincing[2,3] in the case of Messier 32).

Twelve years ago a group at Caltech announced[4] the discovery of a black hole with mass of about 5×10^9 $M_\odot$ at the centre of one of the nearest giant radio galaxies, Messier 87. This galaxy is roughly thirty times further away than Messier 32, so the discovery involved resolving structure only a few arcseconds across. Since that pioneering work a debate has raged as to whether the inevitable uncertainties in such small-scale detail allow the measurements to be interpreted without invoking a central massive object. The jury is still out on this one, although the case for a black hole is now a strong one. Bland-Hawthorne *et al.*[1] have been observing a galaxy six times further away still than Messier 87, and argue that it harbours an even more massive black hole: $M > 10^{10}$ $M_\odot$. Their



The rotation curve for Disk 2 in the double-nucleus galaxy NGC6240. The steepness of the curve could indicate the presence of an ultramassive black hole in the vicinity.

work differs from that of the Caltech group and their successors in focusing attention on the dynamics of gas rather than stars — Bland-Hawthorne *et al.* used a Fabry–Perot etalon spectrometer to determine line-of-sight velocities of the galaxy's hydrogen from doppler shifts in the Hα emission line.

In a quiescent system, gas soon settles to an orderly disk of material on effectively circular orbits and provides the ideal probe of the local gravitational field. (Indeed, it is precisely by studying gas disks around spiral galaxies that astronomers have concluded that galaxies are enveloped in much more massive 'dark halos'.) However, NGC6240, the galaxy studied by Bland-Hawthorne *et al.*, is by no means quiescent. In fact it is generally agreed to be *two* galaxies in collision. In particular, it has a double-humped nucleus and Bland-Hawthorne *et al.* interpret the kinematics of its gaseous component in terms of two disks rotating in different planes.

Disk 1 appears to rotate like many another galactic disk: as one looks further from the nucleus, the rotation speed rises approxi-

mately linearly with radius before flattening off at a value that remains constant as far out as it can be followed. The 'rotation curve' of Disk 2, shown in the figure, is very different. Near its centre the line-of-sight velocity changes by 800 km s^{-1} in 5 arcseconds (about 2.4 kiloparsec). In fact this change may be even steeper than the figure suggests, as Bland-Hawthorne *et al.* could not have spatially resolved a more abrupt change. In any event, if one assumes that material at the peaks of the rotation curve is on circular orbits, one can estimate the mass within 2.5 arcseconds required to hold it on such orbits; nothing less than $4.5 \times 10^{10}\ M_\odot$ will do.

This is, by any standards, a lot of mass in a small space, but what makes it more remarkable is how little light is coming from the same region. This region does not include the galaxy's double-humped nucleus, and even after making generous allowance for absorption of its luminosity by dust, with this mass estimate it contains more than six times as much mass per unit luminosity as a normal stellar population. Thus the straightforward interpretation of the rotation curve in the figure suggests that near the centre of Disk 2 NGC6240 is twice as dark as the famous core of Messier 87. If this darkness is caused by adding a black hole to a normal stellar population, the hole must weigh more than $3 \times 10^{10}\ M_\odot$, much more than any conceivable black hole in Messier 87.

The key question is: can one safely interpret the peaks in the rotation curve as the local speed of a circular orbit? One striking feature of the figure is the extremely rapid decline in the rotation velocity just outside the peak. This decline is faster than the form, $v \propto 1/r^{1/2}$, characteristic of rotation about a point mass. Such a steep fall-off in velocity is most naturally explained in terms of a stellar bar across the middle of the galaxy; the shapes of orbits in a barred gravitational field can change rapidly from elongated to round, and this gives rise to steep gradients in line-of-sight velocities when the system is viewed from certain angles[5]. Furthermore, numerical simulations of mergers[6] show that the old stellar components of merging galaxies tend to develop strong bars whose gravitational fields deprive gas of its angular momentum. The gas then plunges to the system's centre on highly elongated orbits. Could the observations of Bland-Hawthorne *et al.* be neatly explained by such a model without invoking a massive black hole? In any event, the search for these objects must go on. □

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MODEL MEMBRANES

Playing a molecular accordion

Michael Cates

BIOLOGICAL membranes, such as cell walls, are composed mainly of lipid bilayer. In red blood cells, the stability and shape of the membrane is partly controlled by coupling to an adjacent network of polymer molecules (the cytoskeleton), attached to the membrane by anchor groups. The physical properties of purified model membranes, which can be made to form small spherical shells called liposomes, have been studied extensively[1]. Now, Ringsdorf *et al.*[2] have studied the adsorption of a synthetic polymer (with suitable anchor groups) onto liposomes, and have shown that a reversible folding of the attached polymer can occur under changes in temperature; they call their system a "molecular accordion" (Fig. 1). The authors draw a parallel between this effect and the reversible changes of shape that occur in the cytoskeleton of red blood cells during cell motion.

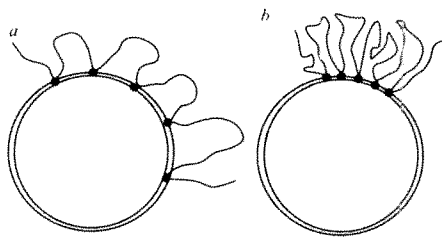

FIG. 1 Adsorbed state of polymer and lipid bilayer. *a*, Good solvent; *b*, poor solvent.

The system the authors studied consists of a high-molecular-weight, water-soluble polymer, poly-*N*-isopropylacrylamide (PNIPAM), to which aromatic side groups (the anchor groups) are attached every 200 monomers or so along the chain. The authors added this to a solution of unilamellar liposomes. The state of adsorption and folding of the chain was monitored by a fluorescence technique, which provides data on what fraction of the anchor groups are close to another such group. The fraction is high when the polymer is dissolved in water (without added liposome) since in this case the polymer forms a micellar structure (Fig. 2) in which the anchor moieties are clustered together to avoid unfavourable contact with water. On addition of liposomes (made of dimyristoyllecithin, DMPC), the fluorescence signal decreases dramatically, indicating that anchor groups are well separated — as with the adsorbed-layer structure of Fig. 1*a*.

The molecular-accordion effect is then brought about by increasing the temperature of the system. At around 32 °C, PNIPAM has a lower critical solution temperature: water is a good solvent for the polymer below this temperature, and an isolated coil of pure PNIPAM (without any anchor groups) will form an expanded structure to maximize its contact with the solvent. At higher temperatures, however, such contacts are unfavourable and the coil collapses. This coil–globule transition is ubiquitous in simple polymers (although in many systems the dependence on temperature is reversed). A similar transition is often seen in proteins, although the chemical heterogeneity of the chain means that the effect is a more complex one. Ringsdorf *et al.* exploit the phenomenon by raising the temperature of the combined polymer–liposome system (Fig. 1*a*). The collapse of the chain causes the anchor groups to move inwards, increasing the number of anchor–anchor contacts and enhancing the fluorescence signal (Fig. 1*b*). The whole process is reversible, so that the adsorbed polymer can be expanded and contracted repeatedly by cycling the temperature — like playing an accordion.

This is certainly a neat experiment, which illustrates an elegant if straightforward piece of physics. But what is its significance for biology? The new results of Ringsdorf *et al.* follow earlier work in which it was shown that the equilibrium shape of a liposome can be strongly altered by adding adsorbed polymer[3,4]. Despite this, there is no evidence in the PNIPAM/DMPC system that the liposomes actually 'dance to the accordion' by undergoing sympathetic changes in shape (the anchor groups could simply slide together within the bilayer, which acts as a two-dimensional liquid). The authors suggest that this might be achieved by choosing larger, more flexible liposomes and by cross-linking the adsorbed polymer into a network. In any case, the numerous shape changes of red blood cells suggest an exquisite dependence of the cytoskeletal protein network on physiological conditions; it would be surprising if such subtlety were related to so gross a conformational change as a coil–globule transition. We do not yet know whether the molecular accordion and the red blood cell cytoskeleton are really playing the same tune. □

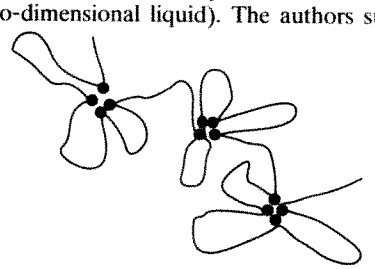

FIG. 2 Micellar state of a free chain containing anchor groups.

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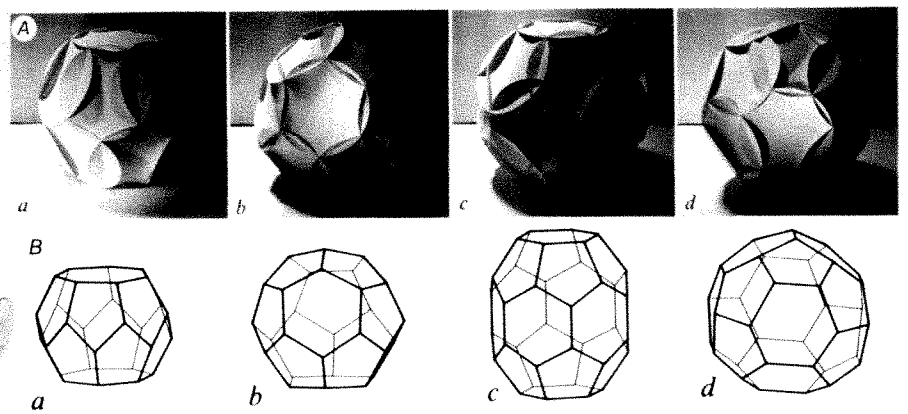
MATHEMATICS

Circularly covering clathrin

Ian Stewart

MATHEMATICIANS often take inspiration from nature, the latest case in point being the problem of covering a sphere by identical circular disks. In *Journal of Molecular Biology*[1], with details to appear elsewhere[2], Tibor Tarnai announces that several arrangements widely conjectured to be the best possible can in fact be improved. The improvements are derived from the observed form of biological structures known as 'coated vesicles'.



A, Cardboard models of the covering of a sphere by disks of equal size. *a* and *b*, covering by 16 disks; *c* and *d*, covering by 20 disks. *a* and *c* are the conjectured optimal solutions for 16 disks[3] and 20 disks[4]; *b* and *d* are the better solutions offered by Tarnai's study of clathrin cages. *B*, Stick polyhedra of equal edges, each of which corresponds topologically to the similarly labelled photograph in *A*. *a*, 'Heptagonal drum' of 2 individual heptagons and 2 rings of 7 pentagons. *b*, Cage polyhedron of 12 pentagons and 4 hexagons, tetrahedrally arranged. *c*, The 'hexagonal barrel', a cage polyhedron of 2 rings of 6 pentagons, 1 ring of 6 hexagons and 2 individual hexagons. *d*, The 'tennis ball', a cage polyhedron of 2 curved strips of 4 hexagons, and 1 space loop of 12 pentagons analogous to the seam of a tennis ball. (Reproduced from ref. 1.)

First, the covering problem. An alien race, the Yuppi, are installing a network of cellular telephones on a distant world. The planet has no open bodies of water, and may be considered a perfect sphere. A certain number of radio stations must be placed on the planet so that every point is within receiving distance of at least one station. Because the Yuppi place great store on cost-effectiveness, and stronger radio receivers are more expensive, they wish to minimize that distance. In more orthodox language, if a sphere is to be covered by a number of identical circular disks, how should they be placed to make their common radius as small as possible?

For small numbers of disks the problem is easily solved. For example one disk can be placed anywhere, and its radius should be half the sphere's circumference, so that it wraps itself round the entire sphere. Two disks should be diametrically opposed, like north and south hemispheres. Symmetric arrangements tend to occur; thus four disks should be placed at the vertices of a regular tetrahedron, and twelve at the vertices of a regular dodecahedron.

The best possible solutions are known only when the number of disks is at most 14 (ref. 3). For greater numbers, conjectured optimal coverings have been published only for 16 disks[3] and 20 disks[4] (see figure, *Aa* and *Ac*).

Enter the coated vesicles. These have an external lattice structure known as clathrin. R. A. Crowther and colleagues[5] noticed that several common clathrin cages possess 12 pentagonal faces, the rest being hexagonal. The same thing happens in the sphere-covering problem. The arrangement of the disks can be represented by a polyhedron whose vertices are placed at, or radially in line with, their centres; and when there are more than 14 disks, the best known coverings all correspond to polyhedra with 12 pentagonal faces, the remainder being hexagonal. In view of this, Crowther *et al.* suggested that clathrin cages might provide optimal solutions to the sphere-covering problem. For example, the conjectured solution for 20 disks is identical to the clathrin cage known as the 'hexagonal barrel' (see figure, *Bc*).

Tarnai investigates this suggestion in three cases: 16, 20 and 32 disks. For 16 and 20 disks it turns out that the clathrin cages do not correspond to the arrangements conjectured to be optimal by mathematicians. Instead, they provide *better* solutions to the sphere-covering problem (see figure, *A*). Thus for 20 disks the improved solution corresponds not to the hexagonal barrel, but to a clathrin cage known as the 'tennis ball'. The angular radius of the circular disks improves from the conjectured 30.5° to 29.6°. For 16 disks the improvement is from 33.5° to 32.9°. But when the number of disks is 32 the clathrin structure does agree with the conjectured optimal solution — the truncated icosahedron or 'soccer ball'.

Although mathematicians often take inspiration from nature, usually they expect it to provide problems, not answers. Not for the first time, nature has beaten them at their own game. □

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PLANETARY ATMOSPHERES

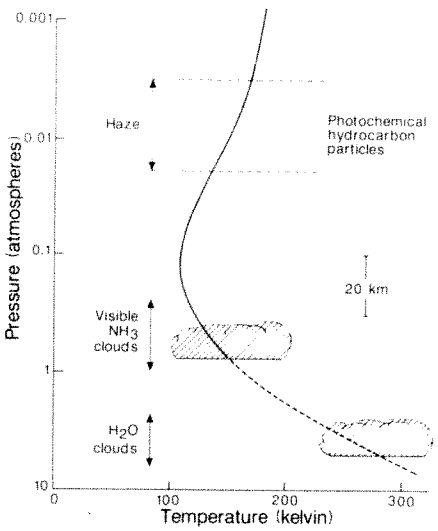
Jupiter's stratosphere mapped

Peter J. Gierasch

OUR knowledge of Jupiter (and the other outer planets) is largely confined to a two-dimensional view at the height of the visible clouds. Motions, patterns, coloration and chemical composition are richly variable at this level, but an understanding of the system will require information from the third dimension. In *Science*, Orton and 14 colleagues[1] present maps of thermal emission from stratospheric levels which will help alleviate the difficulty. The planet was monitored for a decade, a full jovian annual cycle. In spite of the planet's small (3°) obliquity, seasonal effects appear. In addition, there is exciting evidence for variability on a short timescale (only months) which probably indicates upward propagation of disturbances from within or below the visible clouds.

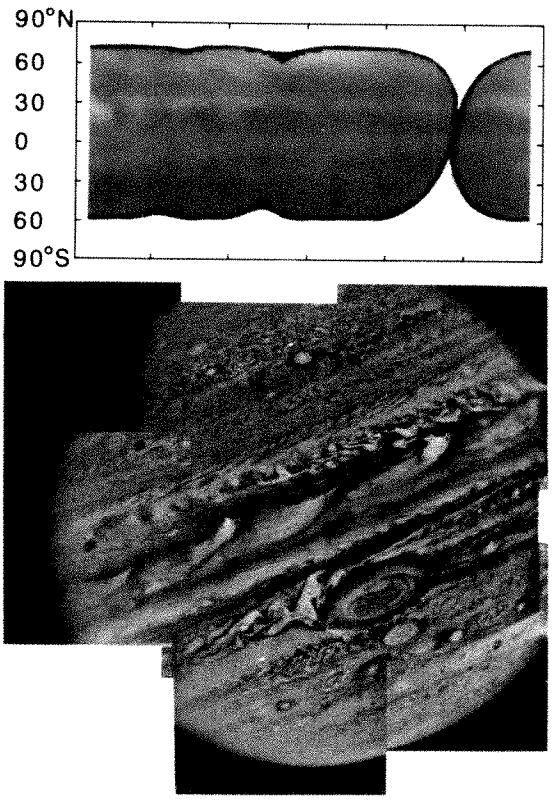
The maps were made at a wavelength of 7.8 μm at the NASA Infrared Telescope Facility on Mauna Kea. At this wavelength, emission is from the middle stratosphere in thermodynamic equilibrium, about 60 km above the visible clouds (Fig. 1). The emitting gas is methane, which is uniformly mixed in the atmosphere of Jupiter. Variations in emission are due to temperature variations in the atmosphere (composition 90% hydrogen, 10% helium and 0.25% methane by number). Horizontal temperature gradients can be produced dynamically by disturbances that penetrate upward from below or by local *in situ* variations in solar heating due to absorption by variable hazes or trace gases. In the latter case the thermal response is governed by a radiative time constant of several years. In the former case fluctuations can be much more rapid, limited only by the wave propagation characteristics of the atmosphere and the rapidity of the excitation processes, which could be as vigorous as terrestrial thunderstorms if they originate in the jovian water clouds, which are very likely to have latent heat effects of the same order as on Earth.

The new data seem to show new phe-



◀ FIG. 1 Vertical structure of Jupiter's atmosphere. The solid line indicates a mean temperature profile as determined by infrared remote sensing. The dashed line is an approximately adiabatic extrapolation through the water clouds, where much dynamical activity is believed to be seated. This level is determined by the water-to-hydrogen ratio (still not well known and probably regionally variable) and the temperature–pressure relation along the adiabat. The maps by Orton *et al.* refer to stratospheric level within the photochemical base. The haze particles are small and do not radiate to any important extent at 7.8 μm, but they absorb sunlight and thereby affect the gas temperature.

FIG. 2 Jupiter in visible light (bottom) and mapped at 7.8 μm (top). A mosaic of Voyager images shows the variety of dynamical activity at the ammonia cloud level on Jupiter. The map of infrared brightness by Orton *et al.* is indicative of the gas temperature about 60 km higher. ▼



nomena of both types. On the slow, seasonal timescale there is a north–south temperature asymmetry that flips sign with the season in the expected manner. In addition, there is an unexpected oscillation at low latitudes whose period is half the orbital period. Two temperature maxima at about ± 20° latitude appear and disappear, symmetrically and in step, during this cycle. The amplitude is approximately 2 K, which is of the same order as the global seasonal contrasts. Since the frequency of this phenomenon is different from that of the direct solar forcing, it must be due to a nonlinear dynamical process, most probably seated in the deep atmosphere.

On shorter timescales the new data are, unfortunately, incomplete — but also tantalizing. The maps show longitudinal structure at a variety of length scales, with a particularly strong signal at a wavenumber of about 11 at a latitude of about 20° N (Fig. 2). The structure varies on timescales as short as a month, and future observations at shorter time intervals will be needed to characterize the changes fully. This surprisingly short timescale (by jovian standards) must mean that these disturbances are attributable to dynamical effects that penetrate all the way from the visible cloud level (or deeper) into the stratosphere. Within the stratosphere itself, there is no obvious way for producing such variability locally: radiative heating is weak, and horizontal temperature gradients are too small to cause instabilities.

On both timescales, the new data point to the equatorial zone, between ± 20 degrees of latitude, as being of special interest to an understanding of the jovian general circulation. This zone includes the 100 m s^{-1} equatorial jet stream and the strong shears at its edges. Previous observations, both in the visible and in the infrared[2], have also indicated particularly vigorous activity in this region. This zone and the Great Red Spot[3] seem to be the two regions on Jupiter where a much needed three-dimensional picture of the circulation might emerge in the near future. Both of the regions are characterized by geometrically large dynamical systems with strong flows and a vertical dependence that may be amenable to observation and analysis. □

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DAEDALUS

Green music

EVER since Darwin tried playing a bassoon to a sensitive plant, the effect of speech and music upon the vegetable kingdom has been a persistent if disreputable area of research. There have even been claims that plants react to musical style — preferring classical to jazz, for example, or absorbing more water when exposed to rock music. Daedalus now has an explanation for such findings.

A green plant, he points out, is a pump. It extracts water from the soil, pumps it upwards as sap, and evaporates it from the leaves. Like all biological fluids, plant sap has a non-newtonian rheology. Accordingly, if placed in an asymmetric opening (such as the stomatal pore of a leaf), and exposed to an asymmetric waveform, its oscillatory flow will not average to zero. It will be pumped either into or out of the leaf. Evolution has presumably arranged matters so that it is pumped outwards by naturally occurring waveforms, such as those generated by the flexing of the leaf in the wind. But a thin, flexible leaf is an ideal microphone. It should also be sensitive to asymmetric sonic waveforms, like the percussive components of rock music.

So DREADCO biologists are exposing various crop plants to asymmetric waveforms, and observing their growth and transpiration rates as a function of waveform and frequency. A plant with large leaves, like rhubarb, will be most affected by low frequencies, while those with small leaves, like beans, will respond better to the upper registers. Elongated leaves, for example those of conifers, wheat and rice, may resonate like quarter-wave aerials to their driving frequencies.

The results should transform agricultural practice. Once the process is understood, loudspeakers in fields and greenhouses will broadcast DREADCO's Green Music to the growing crop. Even by rock standards it will be a boringly monotonous blare. But the plants will love it. With its frequency tuned to vibrate their leaves in resonance, and its waveform matched to the rheology of their sap, it will spur them on to unprecedented growth and vigour.

Weeds, by contrast, could be selectively killed. Natural asymmetric waveforms, produced by impulsive turbulence or mechanical impact, have a sharp 'attack' followed by a gentler decay. But simply by playing a sound backward, modern electronics can easily reverse this state of affairs. The resulting unnatural waveforms should pump the sap back into the leaf, arresting its transpiration. Green Music will include a subtheme of inverted 'killer' tones, lethally tuned to the weeds and causing them to wilt and die. No nasty chemical herbicides will be needed. Ecologists and new-age mystics everywhere will rejoice. David Jones

Mexican site for K/T impact crater?

SIR — A decade ago Penfield and Camargo[1] interpreted gravity and magnetic anomalies from northwestern Yucatan, Mexico, as evidence for a large, buried extraterrestrial impact crater. Research throughout the Caribbean[2–6] suggests that this crater, now named the Chicxulub crater[3], could be the site of the impact purported to have caused mass extinctions at the Cretaceous/Tertiary (K/T) boundary[7]. Using Landsat Thematic Mapper imagery of the Yucatan, we identified[8] a semicircular ring of sink holes, known locally as cenotes, which correlates with the geophysical anomalies noted by Penfield and others[1,3] (Fig. 1). We propose that the origin of the cenote ring is related to post-impact subsidence of the Chicxulub crater rim.

The cenote ring forms a nearly perfect semicircular boundary, 170 km in diameter between unfractured (within the ring) and fractured Tertiary limestones, truncated by the coast and centred 17 km east of Progreso (Fig. 1). This boundary forms a barrier to lateral groundwater migration, causing increased flows, dissolution and collapse along the boundary[9]. Large groundwater flows along the boundary are indicated by a valley-shaped depression in the groundwater surface centred on the ring, and by freshwater springs found where the coastline intersects the ring[8,9]. The cenotes formed by the collapse process are 50–500 m diameter water bodies with depths of 2–120 m. Cenote density and width of the ring vary from about three cenotes per km^2 along a 3-km-wide portion in the southwest (Fig. 2), to a chain of single cenotes 3 km apart in the southeast. This variability is apparently related to differences in the flow of groundwater and fracturing outside the ring.

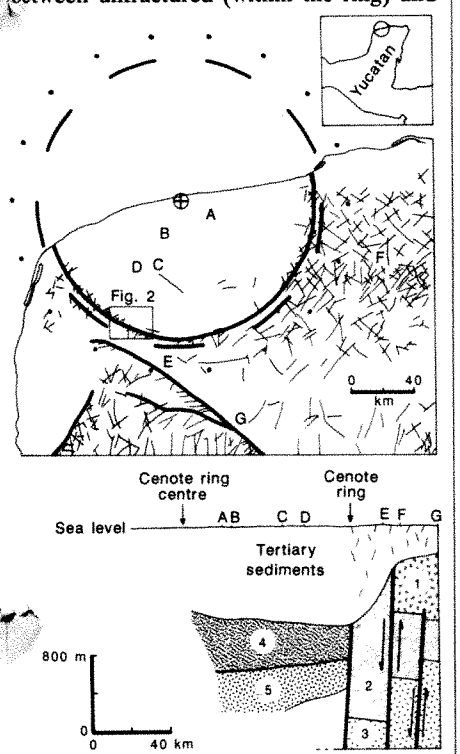

FIG. 1 Structural (upper) and subsurface (lower) geology of the cenote ring, northwestern Yucatan, Mexico (inset). Map fracture traces (thin lines) and faults (thick lines) from ref. 10. Semicircle, cenote ring; dashed circle, approximate location of negative gravity anomaly; dotted circle, approximate outer limit of concentric positive magnetic anomaly. Anomalies from Penfield and others[1,3]. Subsurface data from drill holes are described by Weidie[11] and Lopez Ramos[12], and plotted as a function of the radial distance from the cenote ring centre (hole locations lettered on the map and across the top of the cross-section). Thick lines with arrows show subsidence along possible ring faults; thin lines show fracturing in Tertiary rocks. Key: (1) breccia (ejecta?); (2) Upper Cretaceous marine sediments; (3) Lower Cretaceous marine sediments; (4) breccia (impact?) and crater fill; (5) volcanic rock (impact melt?).

The fracturing that created the cenote ring was almost certainly caused by a circular structure, because no combination of linear stresses would be likely to produce such a nearly perfect circular feature. Except for the fractures, the Tertiary limestones are undeformed, suggesting that the fractures are related to a buried pre-Tertiary structure. A buried impact crater or volcanic caldera could produce a circular structure of this size. We discount the latter possibility because collapse of a caldera would cause fracturing within the ring, and volcanic rocks are found beneath the centre of the ring, not outside as would be expected for a caldera (Fig. 1).

On the other hand, post-impact subsidence induced by slumping and viscous relaxation in the rim of the proposed Chicxulub crater could well have caused the fracturing outside the cenote ring. The magnitude of this subsidence need not have been great to fracture the Tertiary limestones. Viscous relaxation may have been by only metres or tens of metres over the millions of years since the crater was buried. Craters this size have wide or multiple rims, but the fracturing beyond 40 km east and south of the ring is probably related to stresses along adjacent fault systems.

Evidence of subsidence is found in the negative gravity anomaly[3] concentric with and just outside the ring (Fig. 1). Additional evidence of subsidence is the offset of Upper Cretaceous and earlier strata beneath the ring (Fig. 1), which may represent buried ring faults typical of impact crater rims.

FIG. 2 Landsat Thematic Mapper band 5 (infrared) image of a portion of the cenote ring (location shown in Fig. 1). Note chain of cenotes (black dots) across the centre of the image. Width of image, about 31 km. Landsat data from EOSAT Co., Lanham, Maryland, USA.

If there is indeed a crater, the region within the cenote ring corresponds to its floor; the crater rim diameter would then probably be >200 km. If confirmed as a site of impact, the Chicxulub crater would be the largest terrestrial impact crater known, which is consistent with the uniqueness of the Cretaceous/Tertiary global catastrophe.

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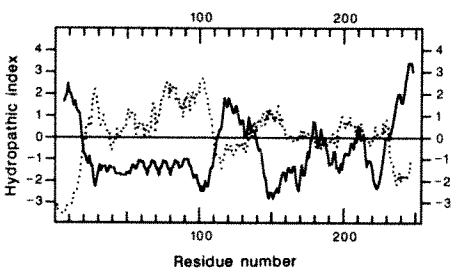
Anticipating the anti-prion protein?

SIR — A host-encoded prion protein (PrP) is crucial in the pathogenesis of transmissible spongiform encephalopathies, such as scrapie and scrapie-like diseases in man and animals[1]. The PrP gene is highly conserved in evolution and contains a single open reading frame (ORF) located within a large exon. Several mutations in the human homologue PRNP gene were found in patients with familial Gerstmann–Sträussler–Scheinker's syndrome (GSS) and Creutzfeld–Jakob disease (CJD).

While analysing PrP complementary DNA sequences with the DNA Strider computer program[2] I found a large overlapping ORF in the DNA strand opposite to the PrP transcriptional unit. The deduced amino-acid sequence of the prospective encoded protein was unique. Its hydropathy plot[3] is almost a mirror image of that of the PrP (see figure). For simplicity the name of anti-PrP is used in reference to this putative protein.

This ORF may be of biological importance first, because it is as large as the PrP ORF. Second, none of the differences in PrP



Hydrophobicity plots[3] of human PrP (solid line) and antiPrP (dot line) proteins generated by the DNA Strider computer program[2]. Numbers on the left and right indicate hydrophobicity index. Numbers above and below indicate amino acid positions in the PrP sequence.

sequences found in patients or animals resulted in additional stop codons in this ORF. It should be noted, however, that mutations found in GSS patients[4,5] in codons 102 and 117 of the PRNP gene produced amino-acid changes in the anti-PrP, whereas mutations found in CJD patients[6,7] in codon 178 and 200 did not. Third, there are ATG or CTG codons at the beginning of this ORF which could serve as translation initiation codons[8]. Fourth, existing data on PrP gene expression do not exclude expression of the anti-PrP gene because only double-stranded DNA probes were used to detect PrP messenger RNA[1]. Obviously, only direct experimental analysis of infected and uninfected cells and tissues with single-stranded probes will show whether the anti-PrP gene is expressed.

The expression of the anti-PrP gene would introduce a new player, present a different view of scrapie infection, and raise new and important questions. Do PrP and anti-PrP proteins interact in infected cells[9]? Do GSS mutations, which, unlike CJD mutations, result in amino-acid changes in both proteins, explain the differences between the two diseases? Do RNA unwindases[10], capable of modifying double-stranded RNA substrates, produce changes in complementary PrP and anti-PrP mRNAs, thus altering the proteins?

In conclusion, the existence of a long anti-PrP ORF clearly warrants a search for the prospective encoded protein, especially in view of its potential role in transmissible spongiform encephalopathies.

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Perceiving depth

SIR — In his News and Views article, (*Nature* **349**, 365–366; 1991) B. J. Rogers comments that B. G. Cummings, E. B. Johnston and A. J. Parker show conclusively (*Nature* **349**, 441–413; 1991) that the human visual system is not able to use vertical disparities in computing three-dimensional shape. But there is more to three-dimensional perception than having two eyes and binocular stereopsis.

The advantages of binocular vision became apparent to me when I was temporarily blind in one eye. With depth perception lost, ordinary household tasks were no problem because I was in a familiar environment, but I did notice difficulty when hanging kitchen implements on hooks at arm's length. The solution was simple. With the object held out towards the hook it was only necessary to turn my head slightly to the side, displacing the apparent relative positions of object and hook, and, in effect, making one eye record the two images normally seen by two for depth to be assessed correctly so the utensils could be hung.

Since reading the two articles I have now checked by closing one eye and repeating the experiment but moving my head up and down slightly to give vertical displacement of the image. The method still works.

In most experiments on vision the visual system is held still. Fortunately, in real life it is not, and practical problems are much easier to solve.

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Oceanic disjunctions

SIR — Long-distance separations (disjunctions) in the geographical distributions of animals and plants are not uncommon[1]. Most result either from long-distance dispersal or from extinction, leaving widely separated relicts. Disjunct distributions between oceanic islands in different oceans are rarer and more surprising. Valdebenito *et al.*'s claim[2] to have found a new one in the disjunction of *Peperomia* (Dicotyledons, Piperaceae) is a little out; this disjunction was first identified by Skottsberg 45 years ago[3]. Groves[4] lists *Peperomia berteroana tristanensis* as "possibly native" to the Tristan da Cunha archipelago in the Atlantic, so the morphological evidence[1] of its distinction from the Juan Fernandez populations in the Pacific is welcome.

Another biogeographic connection between the Tristan da Cunha archipelago and a Pacific archipelago has been known for some time. The subgenus *Trogloscaptomyza* of *Scaptomyza* (Diptera, Drosophilidae) is found only on Nightingale Island in the Tristan archipelago and on several islands of the Hawaiian archipelago[5]. This disjunction was first noted by Hackman[6], who thought it might be a relict distribution. The suggestion that it arose from long-distance dispersal by birds was first made in 1981 (ref. 7), and indeed such dispersal seems to have been important in the spread of *Scaptomyza* round the world from its origin in Hawaii[8].

Other disjunct distributions are known exclusively across oceanic islands. For instance *Trochetiopsis* (Dicotyledons, Sterculiaceae) occurs only on St Helena in the Atlantic (two species) and its closest relative *Trochetia* only on Mauritius and Réunion Islands in the Indian Ocean (six species)[9,10].

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Scientific Correspondence

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Solving a reversed problem

Leonardo da Vinci evolved his reversed handwriting in order to make his scientific notes difficult to interpret. In this way he felt free to express heretical views like "the sun does not move", or describe the deadly weapons he invented. These secrets were so well kept that when Leonardo and his disciples died, nobody could decipher the notes for at least 300 years.

Solving a reversed problem. The information flow in living cells was formerly thought to be one-way only, from DNA to RNA to protein. When it was found that some RNA-viruses could produce a corresponding DNA-chain, the responsible enzyme was named reverse transciptase (RT). Of all the viruses affecting human health only HIV and HTLV are known to be equipped with RT.

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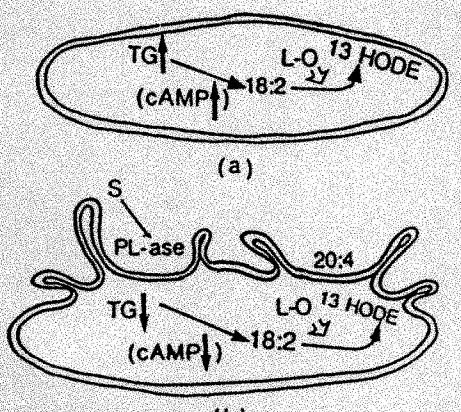

Synthesis of 13-HODE in unstimulated (**a**) and stimulated (**b**) endothelials cells.

The gap between basic thrombosis research and its clinical applications is narrowed in this book. The authors question the assumption that platelets are the predominant factor in the development of atherosclerosis. Indeed, these studies show that monocytes and vessel wall factors may also play an important role. The conclusion is that a new therapeutic approach should aim at influencing these pathways.

G. K. Klintworth

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J. Harenberg, D. L. Heene, G. Stehle, G. Schettler (Eds.)

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Politics and paradox

Fritz Stern

The Kaiser's Chemists. By Jeffrey Allan Johnson. *University of North Carolina Press: 1990. Pp.279. $39.95.*

In the two decades before the First World War, Germany was a country in ascendancy; no state in Europe could match German power, a power and pre-eminence that extended to virtually all fields of national life. It was a dynamic, disciplined society, with a rapidly expanding economy and a scientific establishment that had won the admiration and the envy of the world. That it was also a country in which the modern sectors coexisted with anachronistic political forms is well known; it was a country marked by internal conflicts and anxieties about subversive forces at home and 'encircling' enemies abroad.

Wilhelm II embodied the paradox of his country: convinced of his divine right to rule, insecure and covetous of his people's love and admiration, he was also drawn to the modern — to all that was practical in science and technology. In the field of science, Germany gradually evolved what I would call a state-industrial-scientific complex. There was scientific genius in abundance in that Germany, but genius had to be nurtured; it demanded institutional support — and in turn much of that benefited German industry. The institutional history of German science — a subject that has only recently received the attention it deserves — is intrinsically fascinating and in many respects still pertinent to some of our quandaries today.

The Kaiser's Chemists by Jeffrey Allan Johnson is a curious contribution to this difficult genre. It is a study in two parts: of the failure of three eminent scientists to realize their plans, first advanced in 1905, for an Imperial Institute for Chemistry, and of the success some years later of various groups to establish the Kaiser-Wilhelm-Gesellschaft (KWG) that in turn established the Kaiser Wilhelm Institutes — the first two, opened in 1912, were designated institutes for chemistry and for physical chemistry and electrochemistry.

The failure of the initial effort has received little attention and Johnson's book is most useful in this part. In 1905, the great German chemist Emil Fischer — with the support of two distinguished colleagues, Walther Nernst and Wilhelm Ostwald — sought to mobilize German industry and Reich officialdom in order that an Imperial Institute for Chemistry be established — an analogue to the Imperial Institute for Physics and Technology, created in 1887, at the instigation of Werner Siemens. (See the important work by David Cahan, *An Institute for an Empire: The Physikalisch-Technische Reichsanstalt, 1871–1918*, Cambridge, 1989.) Fischer and his colleagues had experienced in their own careers the difficulties of pursuing basic research at their respective universities: the pressures of teaching were ever increasing, and the resistance of their humanistic colleagues to enlarging the costly scientific sector remained unrelenting. Moreover, Fischer was troubled that Germany's clear lead in organic chemistry was not maintained in the newer and more specialized fields, especially in physical chemistry, with its heavy installation costs. Johnson emphasizes that especially Fischer understood that science was both collaborative and competitive, internationalist and nationalist: the example of new institutions created in the United States — endowed by Carnegie and Rockefeller — was viewed with apprehension in Germany. The chemical industry supported the new plans, but the Imperial Government, somewhat disappointed in the work of the earlier institute, and in any case not directly responsible for education, a realm reserved to the member states of the Reich, declined to cooperate.

Mary Evans

Wilhelm II — eponymous Emperor.

The second half of Johnson's book deals with the more familiar story of the founding of the KWG. It was a different response to the same recognized need, that is, the establishment of institutions where leading scientists could pursue their research unfettered by teaching responsibilities. All manner of plans and promoters coalesced: at the end of his career the Prussian tzar for university affairs, Friedrich Althoff, uncrowned, controversial but relentless in his pursuit of excellence, had hoped to establish a German Oxford on the royal domains in Dahlem — then still a suburb of Berlin. After his death in 1908, it was the liberal theologian and imperial favourite Adolf von Harnack who persuaded the Emperor to lend his name to the establishment of a society that in turn would sponsor semi-independent institutes for research. The Prussian government was miserly in its support, but helped to mobilize philanthropy; a carefully orchestrated 'solicitation' of wealthy individuals — bankers and industrialists — took place, with the tacit understanding that major donors would have a voice in the work of the society and would have the added satisfaction of knowing that they were contributing to a patriotic effort with an imperial nimbus. The campaign to raise sufficient funds would sound familiar to American campaign managers; in the German case, the government discreetly supplied tax information so that the wealthiest could be singled out. Among the wealthy there was a disproportionate number of Jews — and they contributed disproportionately. Membership in the KWG enhanced status and conferred dignity. In 1912, the Kaiser, himself convinced that science and technology were elements of national strength, opened the first two institutes; two years later, responding to the patriotic mood of the day, the director of the Institute for Physical Chemistry and Electrochemistry, Fritz Haber, put his institute at the service of Germany's war effort. In fact it was Haber and some colleagues who persuaded Germany's military leaders that science — through the synthetic production of raw materials that could no longer be imported because of the British blockade, and through the invention of new weapons, most notably and horribly, of poison gas — would prove indispensable to the war effort. Indeed the mobilization of scientific and technological talent allowed Germany to prevail for as long as it did.

Johnson's book is a revised dissertation, originally completed in 1979. It embodies research in German government and business archives; the single most valuable source, it would seem, has been the papers of Emil Fischer, deposited in Berkeley. He acknowledges his debt to two earlier scholars. Lothar Burchardt and Günter Wendel, a scholar in what used to be the German Democratic Republic, who could exploit and perhaps sequester some of the great archival

material that was at his disposal. Today, Johnson's work on the founding of the KWG has been largely superseded by a comprehensive work on the KWG and the Max Planck Society, a work by many hands, including Johnson's (and mine): *Forschung im Spannungsfeld von Politik und Gesellschaft. Geschichte und Struktur der Kaiser-Wilhelm/Max Planck Gesellschaft*, edited by Rudolf Vierhaus and Bernhard von Brocke (Stuttgart, 1990).

Johnson's well-researched book is marred by his relentless wish to give it an all-embracing theoretical framework — hence the constant refrain that all that he details is evidence of Germany's "conservative modernization". On the one hand, there is nothing strikingly new about the thesis that nineteenth-century Germany assimilated modern institutions into an existing, largely authoritarian culture. But what other models for modernization did Johnson have in mind? Radical or revolutionary modernization? His efforts to ground this much-vaunted theory in historical facts, to link the institutional history of science to actual politics leads over and over again to the "fallacy of misplaced concreteness". One example — a central one — must suffice: it is true that Wilhelm II's prestige was damaged by the *Daily Telegraph* affair of 1908, but there is little if any evidence that this celebrated instance of his political ineptitude hastened his support of an enterprise that trusted advisors had submitted to him and that would have corresponded to his more rational impulses in any case. The author would have done better to make more plausible connections between particular facts and rely less on an abstruse and pretentious theoretical framework. It is also regrettable that a book about scientists — many of whom had a superb command of language — should make so little effort at literary distinction.

Johnson rightly emphasized the importance of American developments in German eyes. Scientists sensed the new rival, whereas many humanists lamented the possible intrusion. Johnson quotes from the great reactionary classicist, Ulrich von Wilamowitz-Moellendorff, who in defending the — unthreatened — position of the Prussian Academy of Sciences blames the Kaiser Wilhelm Institutes for having been largely financed by industry: "We cannot blame industry for that, but it is very American." In 1903, Fritz Haber had caught the modernizers' mood when he wrote after a visit to the United States: "The American [economic] challenge has become a common slogan, and Bismarck's sentence about the Germans who fear no one but God would seem in business circles gradually to be seriously amended: and a little the United States." *The Kaiser's Chemists* confirms that German ambivalence about America has a long and important history. □

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Groping in the dark

Murray Stewart

Biophysical Electron Microscopy. Edited by P. W. Hawkes and U. Valdre. *Academic: 1990. Pp. 517. £61.50, $132.*

Most microscopists, especially biological ones, like doing it in the dark, and it is not clear how welcome turning on the lights would be. Groping in the dark is not without its attractions and rewards. But for those who have tired of some of the more mundane pleasures, or who are stimulated by a detailed knowledge of the intricacies of the subject, *Biophysical Electron Microscopy* should provide hours of instruction and even pleasure.

As is traditional in such manuals, it opens with a detailed description of the equipment and its function. It is often amazing to discover some of the misconceptions harboured in this area, even by those thought to be quite experienced, and so a thorough grounding is invaluable. Basic anatomy focuses on the column, but gives a frank and useful discussion of a number of exotic variations as well as the more straightforward methods used by most. Chapters on instrumentation, electron-specimen interactions, image formation and contrast provide a valuable introduction that is often not found in books directed towards a biological audience. High resolution is discussed in detail and gives an insight into the sorts of new information that can be obtained using electron crystallography, while also introducing many of the problems that remain in this specialized field.

A monumental chapter on image processing is probably unrivalled in the literature in terms of thoroughness and rigour. This chapter alone would make the book invaluable for workers interested in a more quantitative view of structural biology. Radiation damage is treated in considerable detail and identifies many of the problems that stem from this area. Low-temperature techniques are treated a little superficially, but do give some good pointers to more specialized sources. Finally, a number of accessory techniques for determining composition (X-ray microanalysis, electron energy-loss spectroscopy, cathodoluminescence) are described.

Although undoubtedly valuable, the rather mathematical emphasis of this book may sometimes make it rather demanding on most biologists. At times, the expectations of the intended audience in terms of matrix algebra or even calculus may be exceeded and some areas would be more valuable with a greater emphasis on explaining basic principles in simple terms rather than only as equations. My impression is that 'biophysicist' is interpreted as physicist looking at biology rather than as a biologist using physical methods to look at the living world.

A major problem for many biologists has been that most books on electron microscopy deal with it from the perspective of materials science. Clearly, a book with a distinctly biological perspective would be extremely valuable. Consequently it is with some considerable sense of frustration that one finds that so many chapters of the present book deal mainly with specimens, examples and problems drawn from materials science. Electron-diffraction patterns from silicon single crystals or AuZn alloy or high-resolution lattice images of gold are of very limited usefulness to biologists. Such materials-science objects differ in a number of significant ways to biological material, most particularly in terms of radiation damage, contrast and the relative importance of dynamic effects. The reason that biologists do electron microscopy differently is far more related to the differences between specimens than to any lack of knowledge about the techniques concerned. In fact, the apparent lack of appreciation by some of the authors of the differences between biological and materials-science objects seriously limits the usefulness of this book. Although at least most of the background information is collated into one place (rather than scattered over several books on materials science), a real opportunity to discuss these points from a biological perspective has been lost.

The excitement of modern structural biology often seems lacking from this book. Macromolecular organization is one of the secrets of life and to most of its practitioners, biophysical electron microscopy is exciting because of the unique insights it can give into this area. The book is depressingly short of examples of the achievements of these techniques and where the subject is heading, and I doubt that a physicist would be enthusiastic about entering biology after reading it.

But notwithstanding these criticisms, *Biophysical Electron Microscopy* is a valuable background reference and will be an indispensible source book for anyone seriously interested in structural biology at anything other than the morphological level. Although it suffers from the usual problems of cohesion and emphasis common to most multi-author volumes, it does combine a very substantial amount of diverse and extremely useful fundamental information about electon microscopy and its underlying physical principles. It is not so much a question of whether it could have been done better, but rather that it is a very substantial achievement to have done it at all. It may well be that for some, in addition to telling them all they ever wanted to know about electron microscopy, it also tells them why perhaps they were quite correct to be afraid to ask. It may well not prevent biologists from groping in the dark, but they will at least have a better idea of how to do it. □

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No holds barred

Guy T. Emery

Science and Cultural Crisis: An Intellectual Biography of Percy Williams Bridgman (1882–1961). By Maila L. Walter. *Stanford University Press: 1990. Pp.362. $42.50.*

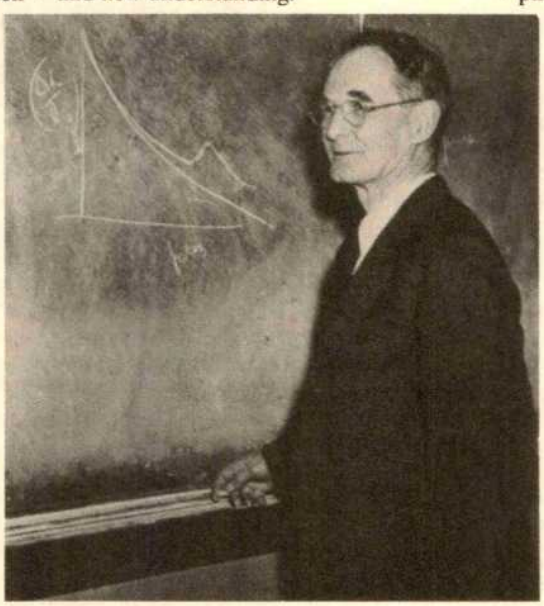
PERCY Williams Bridgman (1882–1961) was an unusually productive and unusually perceptive physicist who made a strong impression on the scientific community. "Brilliant, intense and dedicated" were the adjectives with which his colleagues led off their biographical memoir. How to compress substances under very high physical pressure, and how matter behaved under such high pressure, was his experimental field.

Maila L. Walter's *Science and Cultural Crisis* provides a detailed discussion of Bridgman's life and career. It is based on extensive work on Bridgman's papers and will reward the interest wide range of readers.

From the age of 18, Bridgman found a home at Harvard, as student, scholar and faculty member. As a graduate student he found a way to achieve hydrostatic pressures higher than previously possible. Leaking was eliminated by "a method of packing . . . which automatically becomes tighter the higher the pressure." He used his technical skill to measure several mechanical, electrical and thermal properties of a wide variety of materials over the new ranges of pressure made available, surmounting the challenging problems of calibration with ingenuity and perseverance. His work was careful and precise and was described in prose that, like his life, was straightforward and vigorous.

Approximately 200 scientific papers appeared between 1909 and 1958, later gathered in seven volumes by Harvard University Press. (*Collected Experimental Papers*, 1964). Many of the papers contain extensive data on several different materials. The physics of materials under high pressure, as of the 1950s, was a field created by and dominated by Bridgman.

He worked alone, or with the help of a laboratory technician and a master machinist. The graduate students he supervised — never more than two at a time — pursued their own separate projects. He guarded his time for experimental work by negotiating his freedom from faculty committees, and taught, for most of his years, only graduate courses. In his modest rooms in the basements of Jefferson and Lyman Laboratories Bridgman laboured hard, with his mind and with his body, in pursuit of new data and new understanding.



Bridgman — "Brilliant, intense and dedicated".

The summers Bridgman spent in Randolph, New Hampshire, in a grand sloping valley in the White Mountains. He hiked, raised vegetables, read and played music with his family, and wrote up the accounts of his experiments. He also pondered and wrote about some of the deeper questions raised by physics and how physics is done. An examination of Tolman's attempt to exploit scale-invariance arguments led Bridgman into dimensional analysis, on which he published a book in 1922. (*Dimensional Analysis*, Yale University Press). A more general investigation of the 'meaning' of the conceptual terms used in physics, mostly stimulated by the special theory of relativity but with the interpretative puzzles of quantum mechanics also in mind, resulted in *The Logic of Modern Physics* (Macmillan, 1927), in which he proposed that "we mean by any concept nothing more than a set of operations; *the concept is synonymous with the corresponding set of operations.*" (Bridgman's emphasis).

Bridgman's operational analysis struck a responsive chord among many scientists, and among quite a few philosophers as well. He was very much an individualist, however, and could not be subsumed even by the Vienna Circle of philosophers, with whose approach his own had much in common. He continued to explore these deeper questions, in articles, and in books like *The Nature of Physical Theory* (Princeton University Press, 1936), *The Nature of Thermodynamics* (Harvard University Press, 1941), and *The Way Things Are* (Harvard University Press, 1959).

Some psychologists found operationalism particularly congenial, and through the efforts of S. S. Stevens applications and elaborations of Bridgman's line of thought played a considerable role for some time in that field.

If from Walter's book it seems to a non-philosophical reader that Bridgman's *sui generis* approach to deep questions is sometimes too much nibbled at for not conforming to comfortable philosophical classifications, there is recompense in the realization that his contributions are considered worth so much and so detailed an analysis.

Walter successfully covers the things Bridgman did that make him a culture hero to physicists. For example, she reminds us of his dictum that "The scientific method . . . is . . . doing one's damnedest with one's mind, no holds barred" (*Reflections of a Physicist*, Philosophical Library, 1950); and of the manifesto once posted on his door: "I have decided from now on not to show my apparatus or discuss my experiments with the citizens of any totalitarian state." He did his best to maintain in himself and promote in others the highest standards of intellectual integrity.

Despite his rigour, he could be kind, for example to the young. In the strength and purity of his intellectualism he was not unlike Wittgenstein, with whom he shared a disquietude about the diagonal proof of non-denumerability. His operationism was in one sense a pointing at what scientists do, as more powerful than categorization by words.

In his later writings Bridgman stressed the privateness of science, and of human life itself. "Beyond the public level, waiting for a deeper analysis, is the private level. It is on the private level that I realize my essential isolation; here is my awful freedom that I can hardly face" (*Reflections*, p.75). Walter is good on this important existential strain in Bridgman.

Into his middle seventies Bridgman rode a bicycle to work. When faced by rapidly spreading inoperable cancer his response was as stoic, rational and straightforward as the rest of his life: he put the index to his collected scientific papers in the mail, wrote a brief note, and shot himself. Bridgeman's life and his writings had a deep effect on physicists. Walter's book is a good reminder of this. □

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Cells evolving

T. Cavalier-Smith

Blueprint for a Cell: The Nature and Origin of Life. By C. de Duve. *Patterson/Portland: 1991. Pp.275. $19.95, £17.95.*

FOR almost 3,000 million years living organisms were essentially unicellular: all evolution was cell evolution. But, curious though it may seem, much less has been written about the evolution of cells than about the relatively brief chemical evolution that preceded it or the recent evolution of multicellular organisms. De Duve's attractive and well-written book, *Blueprint for a Cell*, is therefore most welcome. The author is best known for 'discovering', or rather establishing the true nature of, lysosomes and peroxisomes. His earlier *A Guided Tour of the Living Cell (Scientific American Books, 1984)* was one of the best semipopular introductions to cell biology. The present book also deserves to be widely read.

Its first part outlines the most essential features of living organisms and how these are differently expressed in bacterial and eukaryotic cells. This leads to a reconstruction of the ancestral cell, in preparation for the second part which attempts to suggest how this ancestral cell may have originated. Part one is a rather well-balanced treatment that generally avoids excessive bias in one or other fashionable directions. But it does somewhat overemphasize the living state, at the expense of the processes of cell reproduction, which are absolutely central to an understanding of the dynamics of cell evolution.

The chapter on bacteria does not even mention the vital role of the rigid cell wall in cell growth and division and in providing a rigid framework for the segregation of DNA. Therefore the drastic implications of its loss for the origin of the eukaryotic cell are only partially appreciated. When discussing the origin of mitosis de Duve suffers from a widespread misconception, often propagated in even the best textbooks, that dinoflagellate chromosomes are not attached to spindle microtubules, and that segregation by membrane growth was a primitive eukaryote condition. In fact there is no reason to think that fluid lipid bilayers are able to segregate DNA in any organism. Some sort of skeleton, whether an exoskeleton as the bacterial wall or an endoskeleton as the eukaryote cytoskeleton, are essential for the reliable transmission of genetic information from generation to generation. The changeover from the bacterial to the eukaryotic pattern must have been far more traumatic than de Duve recognizes.

The whole book suffers from a gradualistic and a deterministic bias. The author does not recognize the evolutionary importance of historical contingency or of long periods of stasis followed by sudden change. Far too little note is taken of the fossil record, which suggests that eukaryotes evolved only about half as long ago as bacteria (1.7×10^9 years ago, not $3.5–2.3 \times 10^9$ years ago as the author implies), and in no way supports the author's view that the origin of eukaryotes and of ourselves was inevitable. To dismiss chance and historical accidents as miracles and to allow only strict determinism is philosophically unsound.

Although the author has been overinfluenced by uncalibrated gradualistic 'molecular-clock' ideas in his dating, he rightly realizes that the most recent common ancestor of all life cannot possibly have been a 'nebulous' progenote, but must have been a highly developed bacterial cell. He criticizes my view that this was eubacterial—not archaebacterial—in its lipids because he thinks that acylester lipids could have been invented twice; yet he inconsistently invokes possible biophysical reasons to explain why in archaebacteria a changeover from isoprenoidalether to acylester lipids never occurred. If it never occurred for such reasons in archaebacteria, why postulate it twice independently in eukaryotes and eubacteria? I am tempted to conclude that de Duve's reluctance to accept that the ancestral cell was a eubacterium is because he prefers to suppose it was an acid-resistant, thermophilic sulphur-dependent archaebacterium, because this appears to bolster his dubious theory for the prebiotic origin of a bioenergetic system based on thioesters.

In contrast to the first part of the book which is basically sound, part two is wildly speculative and fundamentally flawed in its two central theses. The most fundamental of these is the idea that chemical evolution produced a complete protometabolism of metabolic pathways catalysed by noninformed oligopeptides before the origin of replication and translation; moreover this protometabolism, which is incredibly held to have included essentially all the currently existing biosynthetic pathways and therefore all the raw materials for life, was preserved in some way during the changeover from uninformed to coded protein synthesis. Neither half of this central thesis is remotely plausible. Yet in one sense this general line of thinking ought to be pursued, and studied experimentally, because it must surely be the case that replication and translation originated, not in almost pure systems of the sort currently studied in the laboratory but in incredibly complex mixtures of molecules produced by the factors (mass action and crude prebiotic catalysts) that de Duve so lucidly describes. Even if, contrary to de Duve's hopeful assertions, this 'protometabolism' did not in fact give rise directly to modern metabolism it might well, as he argues, have provided an essential prerequisite for the origin of the constituents of the 'RNA world' or some yet more primitive self-replicating system.

De Duve's second thesis is that his protometabolism evolved a complete bioenergetic system that was initially based on thioesters, and later evolved substrate-level phosphorylation, all before the origin of replication, translation or the first cell. He rejects the more conventional idea that bioenergetics started with pyrophosphate, because of the low concentration of phosphate at neutral pH in the presence of calcium. But because his thioester theory also would not work at neutral pH and low temperatures, he suggests that life began not at neutral pH but in hot acid (despite the instability of many key biogenic molecules under these conditions). But if one can postulate special environmental conditions for thioesters why not also for phosphates? De Duve does not explain why the low concentration of phosphate was not equally a problem for the postulated later origin of phosphorylation and nucleotides. His reasons for rejecting the widely discussed idea that the first bioenergetic system was a membrane-based photophosphorylation (not substrate-level phosphorylation) are equally unclear: perhaps it simply reflects the traditional biases of animal biochemists, which de Duve openly admits he shares.

In much of part two the selective forces that favour each postulated stage are very vague and inexplicit. For example, when discussing the origin of translation he says that it was not "the quality of the messages that counts", but of the ability to synthesize polypeptides. But though selection can directly improve replication regardless of its other phenotypic consequences this cannot be true of translation: there must be some specific benefit of the product to the system.

De Duve recognizes the importance of the association of early replicating molecules with some structure, for example a membrane, that can itself grow and divide and therefore be subject to 'true darwinian selection' but is very vague as to the specific benefits of such association: if the first bioenergetic system depended on membranes, as in the phototrophic theory, the advantages are by contrast quite obvious. He favours the inside-out cell/obcell of G. Blobel and myself as a precursor for true cells, but is very vague as to the properties of his postulated protocell: in one place he assumes it was porous and in another impermeable to protons.

Even though he touches on many of the most important considerations for understanding the origin of cells there is relatively poor integration in part two between them, which is a pity because the subject of cell evolution (as opposed to the more specialized and limited molecular evolution) should above all else be integrative one. Nonetheless, though to some degree falling between two stools (too technical for the intelligent layman; insufficiently detailed or rigorously argued for the specialist), *Blueprint for a Cell* will be stimulating reading for a broad scientific audience.

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Quantum optical tests of complementarity

Marlan O. Scully, Berthold-Georg Englert & Herbert Walther

Simultaneous observation of wave and particle behaviour is prohibited, usually by the position–momentum uncertainty relation. New detectors, constructed with the aid of modern quantum optics, provide a way around this obstacle in atom interferometers, and allow the investigation of other mechanisms that enforce complementarity.

COMPLEMENTARITY distinguishes the world of quantum phenomena from the realm of classical physics. The lion's share of the credit for teaching us to accept complementarity as a fact and for insisting that we have to learn to live with it belongs to Niels Bohr. In 1927, when he was reviewing the subject at Como[1] in a speech delivered in honour of Count Alessandro Volta (1745–1827), quantum theory as we know it today was still new, and all examples used to illustrate complementarity referred to the position (particle-like) and momentum (wave-like) attributes of a quantum mechanical object, be it a photon or a massive particle. This is the historical reason why complementarity is often superficially identified with the 'wave–particle duality of matter'.

Richard Feynman, discussing the two-slit experiment in his admirable introduction to quantum mechanics[2], notes that this wave–particle dual behaviour contains the basic mystery of quantum mechanics. In fact, he goes so far as to say: "In reality it contains the only mystery."

Complementarity, however, is a more general concept. We say that two observables are 'complementary' if precise knowledge of one of them implies that all possible outcomes of measuring the other one are equally probable. We may illustrate this by two extreme examples. (A more general discussion is given in ref. 3.) The first example consists of the position and momentum (along one direction) of a particle: if, say, the position is predetermined then the result of a momentum measurement cannot be predicted and all momentum values are equally probable (in a large range). The second extreme involves two orthogonal spin components of a spin-1/2 particle: if, say, the vertical spin component has a definite value ('up' or 'down') then upon measuring a horizontal component both values ('left' or 'right', for instance) are found, each with a probability of 50%. Here then is the 'Principle of Complementarity':

> For each degree of freedom the dynamical variables are a pair of complementary observables.

A less formal, less precise version in practical terms is:

> No matter how the system is prepared, there is always a measurement whose outcome is utterly unpredictable.

Thus, in the microcosmos complete knowledge of the future in the sense of classical physics is not available. This, in essence, is the 'mystery' pointed to by Feynman.

As is true for all physical principles, the actual mechanisms that enforce complementarity vary from one experimental situation to another. Over the years various gedanken experiments have been analysed which emphasize this complementarity in quantum mechanics. Examples include Albert Einstein's recoiling-slit arrangement[4] (analysed in the spirit of Willis Lamb[5] in ref. 6), Feynman's electron–light scattering scheme[2] and Werner Heisenberg's microscope[7]. In the first two of these examples Heisenberg's position–momentum uncertainty relation[8]

$$\delta x \delta p \geq \frac{\hbar}{2} \qquad (1)$$

makes it impossible to determine which hole the electron (or photon) passes through without at the same time disturbing the electrons (photons) enough to destroy the interference pattern. Similar conclusions are reached by Heisenberg in his classic microscope example.[7] In the present work we have found a way around this position–momentum uncertainty obstacle.

That is, we have found a way, based on matter-wave interferometry, and recent advances in quantum optics, namely the micromaser[9–14] and laser cooling[15,16], to obtain which-path or particle-like information without scattering or otherwise introducing large uncontrolled phase factors into the interfering beams. To be sure, we find that the interference fringes disappear once we have which-path information, but we conclude that this disappearance originates in correlations between the measuring apparatus and the systems being observed. The principle of complementarity is manifest although the position–momentum uncertainty relation plays no role.

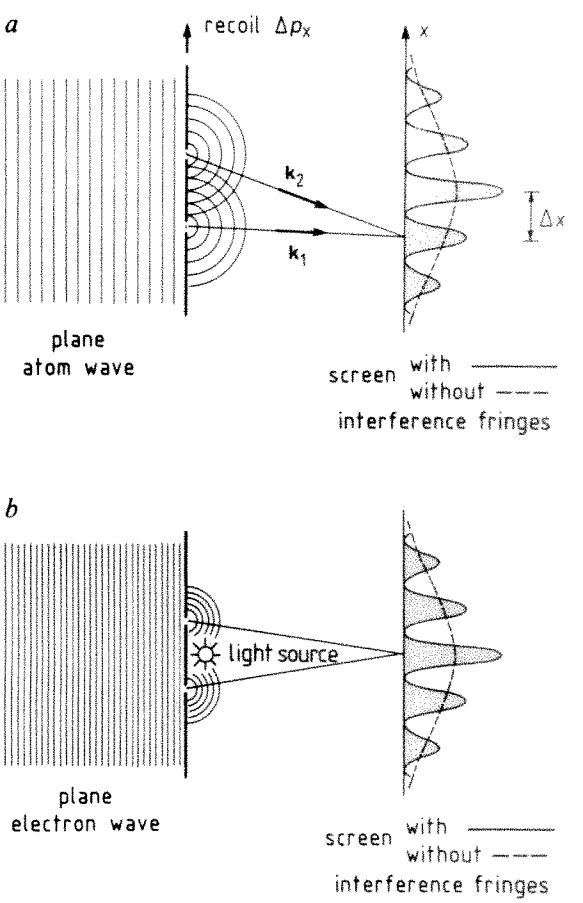

FIG. 1 *a*, Einstein's variant of the two-slit experiment. In this gedanken experiment the slits can recoil and reveal through which slit the photon reached the screen, inasmuch as only one of the wave vectors $\mathbf{k}_1$ and $\mathbf{k}_2$ is consistent with a known amount of recoil momentum. *b*, Feynman's version of Einstein's gedanken experiment. Here electrons interfere, and the scattering of photons is used to detect their position just behind the slits, revealing through which slit the electron reached the screen.

Gedanken experiments illustrating complementarity

We now turn to a brief survey of the usual textbook examples. (As a recent textbook we recommend ref. 17.) These examples traditionally involve a two-slit experiment in which light is allowed to interfere on a distant screen, thereby showing interference phenomena, the hallmark of wave-like behaviour. However, if we are able to detect which path the light has followed, we have particle-like information, and Nature refuses to let us observe wave-like phenomena.

Perhaps the archetypal example is Einstein's recoiling-slit arrangement[4,6], depicted in Fig. 1*a*. Einstein hoped, by this example, to give a gedanken experiment which would yield both which-path (German: *welcher Weg*) information and also show wave-like interference phenomena. But Bohr[4] pointed out that we must also treat the recoiling slits by the laws of quantum mechanics. As discussed in Box 1, one then learns that there cannot be an interference pattern if the experiment allows us to determine through which slit the photon reached the screen.

In another example along these lines Feynman[2] replaces the photons by electrons. As the wave nature of matter is well known, interference between the electrons passing through slits, as in Fig. 1*b*, would be expected to lead to the usual fringe pattern on the screen. (Indeed, precision experiments, in which slow neutrons with a de Broglie wavelength of ~20 Å pass through two macroscopic slits with widths of ~150 μm demonstrate perfect agreement with the quantum mechanical predictions; see Fig. 2.) In this scheme we now have an extra 'handle' on the interfering particles as electrons can be observed by, for example, light scattering. This is depicted in Fig. 1*b* where we see a light source which would scatter light from the vicinity of either slit depending on which slit the electron comes through. Feynman then goes on to explain that this observation procedure destroys the interference pattern as seen on the screen. He concludes his analysis of this interesting example with the following statement:

> If an apparatus is capable of determining which hole the electron goes through, it *cannot* be so delicate that it does not disturb the pattern in an essential way. No one has ever found (or even thought of) a way around the uncertainty principle.

In the experimental situations discussed so far, as in all standard examples, including Heisenberg's famous microscope[7], complementarity is enforced with the aid of Heisenberg's position–momentum uncertainty relation. Is this mechanism always at work? No! We have recently[18–20] found a way around it.

We have developed and analysed a scheme that is very much in the spirit of Einstein's original proposal: we observe which path the particle has followed and do this without appreciably altering the spatial wave function. If we can do this, we will have shown that Einstein's goal is realizable, and the question of how the principle of complementarity is enforced must then be readdressed. Here we will show that Einstein's goal is indeed obtainable: it is possible to obtain *welcher Weg* information without exposing the interfering beams to uncontrollable scattering events.

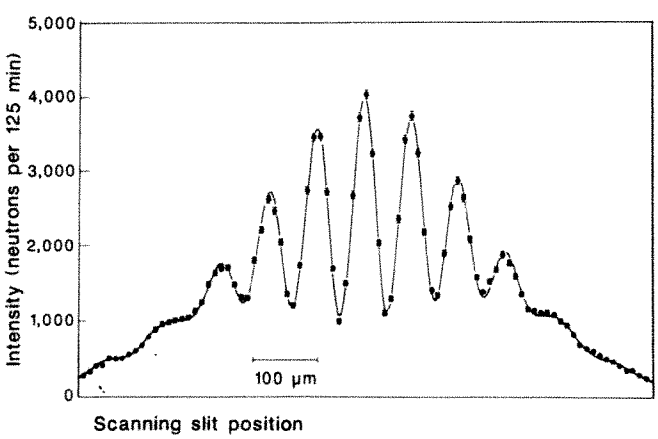

FIG. 2 Interference pattern produced by slow neutrons passing through a double slit. The solid curve represents the quantum mechanical prediction without any fitting. This plot is taken from ref. 34.

On the other hand, Bohr would not have been distressed by the outcome of these considerations, as wave-like (interference) phenomenon is lost as soon as one is able to tell which path the atom traversed. Quantum mechanics contains a built-in safeguard such that the loss of coherence in measurements on quantum systems can always be traced to correlations between the measuring apparatus and the system being observed. That is to say, in the present example, it is simply the information contained in a functioning measuring apparatus that changes the outcome of the experiment, and not uncontrollable alterations of the spatial wave function, resulting from the action of the measuring apparatus on the system under observation.

These considerations are based largely on recent advances in the field of quantum optics, in particular the development of micromaser techniques[9–14]. In these experiments one can ensure that an atom passing through a cavity will make a transition from an excited state to a lower state because of the interaction with the photons in the cavity.

To appreciate the logic of the present scheme, let us consider a beam of atoms replacing the light beam in the Einstein–Bohr dialogue, and the electron beam in the Feynman example. Just as in the previous cases, a beam of atoms incident upon a two-slit arrangement will show an interference pattern. As indicated in Fig. 3, a series of wider slits is used as collimators to define two atomic beams that arrive at the narrow slits where the interference pattern originates.

Let us disregard for the moment the laser and the maser cavities indicated in Fig. 3. In the interference region, the wave function describing the centre-of-mass motion of the atoms is then the sum of two terms referring to the two slits

$$\Psi(\mathbf{r}) = \frac{1}{\sqrt{2}}[\psi_1(\mathbf{r}) + \psi_2(\mathbf{r})] \qquad (2)$$

and the probability density of particles falling on the screen where $\mathbf{r} = \mathbf{R}$, denoted by $P(\mathbf{R})$, will be given by the squared modulus of $\Psi(\mathbf{R})$, that is

$$P(\mathbf{R}) = \tfrac{1}{2}[|\psi_1(\mathbf{R})|^2 + |\psi_2(\mathbf{R})|^2 + \psi_1(\mathbf{R})^*\psi_2(\mathbf{R}) + \psi_2(\mathbf{R})^*\psi_1(\mathbf{R})] \qquad (3)$$

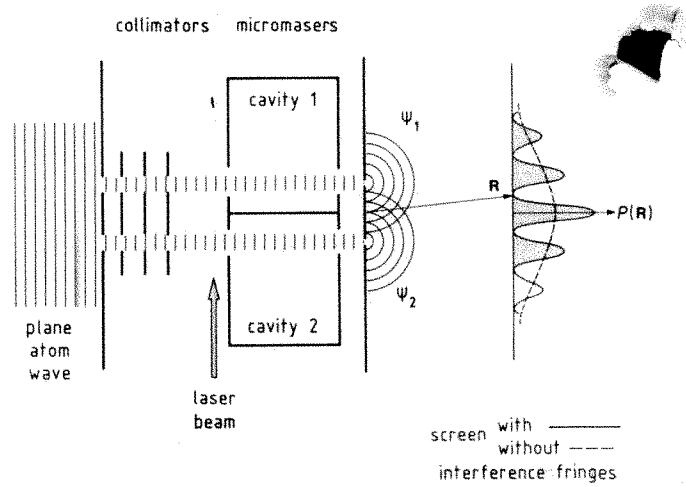

FIG. 3 Two-slit experiment with atoms. A set of wider slits collimates two atom beams which illuminate the narrow slits where the interference pattern originates. The collimation of the atomic beams would actually be done using atomic optics. One could, for instance, employ six-pole fields operating either on the magnetic dipole moment, or in the case of Rydberg atoms on the field-induced electric dipole moment. This set-up is supplemented by two high-quality micromaser cavities and a laser beam to to provide which-path information.

We note that the usual interference behaviour is represented by the cross-terms $\psi_1^*\psi_2 + \psi_2^*\psi_1$.

Now just as Feynman's electron beam provided us with another handle on the interfering particles, not present in the case of interfering light beams, so, in the present case of an atomic system, we have further degrees of freedom involving the internal structure of the atom that are not available to us in the electron beam example.

In fact, we can now envisage preparing the atomic beam in an excited state (with the aid of a suitably operated laser) and then allowing the atoms to pass through the maser cavities in Fig. 3. On traversing either one of the cavities, the atom will emit a microwave photon and could leave *welcher Weg* information in the cavity.

One might think that the process of interacting with the microwave cavity fields and spontaneously emitting photons would disturb the centre-of-mass wave functions $\psi_1(\mathbf{r})$ and $\psi_2(\mathbf{r})$. The careful calculation reported in ref. 20 shows that this is not true. The natural way to discuss the centre-of-mass motion (the only essential parameter to be considered here) is in terms of kinetic and potential energy. In this language, the coupling between the atom and either one of the quantized cavity fields appears as a very small potential energy, whose sign and magnitude depends on the internal atomic and photonic quantum numbers. The wave function then consists of two components, one exposed to a weak attractive potential and the other to a repulsive one; the dynamical difference between attraction and repulsion effects the internal atomic transition accompanied by the emission of a photon. After the atom has traversed the cavity, it is again in force-free space and its momentum has the initial value. Thus, no net momentum is transferred to the atom during the interaction with the cavity fields. The de Broglie wave length of the atom is, therefore, not affected when a cavity photon is emitted, and so we have here an experiment which is "so delicate that it does not disturb" the interference pattern. (We should mention here that it is not possible to associate a definite momentum with a cavity mode, as this is not defined for a localized photon. Therefore the discussion of the atom–field interaction cannot be carried out on the basis of momentum transfer.)

In this sense we have conceived a *welcher Weg* detector which does not fall prey to the position–momentum uncertainty relation. How then are we to deal with the issue of complementarity? As discussed in the next section, it is simply the correlations between the detectors (micromaser cavities) and the atomic beams which are responsible for the loss of coherence (interference fringes) in the present experimental arrangement.

The above discussion of the *welcher Weg* detector was based on an atomic interference experiment. We should mention here that other experimental arrangements are also possible. For example, the two-field method developed by Norman Ramsey[21] can be applied. If the two fields necessary for this method are produced in identical micromaser cavities which are being traversed by the atoms one after the other, then equation (3) applies as well. The quantum-beat experiment proposed in ref. 19 is another possible scheme.

In the next section the micromaser *welcher Weg* detector is studied in more detail. In the section after next, we then ask what happens if one erases the which-path information contained in the *welcher Weg* micromaser cavities. Will interference fringes reappear?

A micromaser *welcher Weg* detector

A key ingredient in the micromaser *welcher Weg* detector is an excited atom which emits a photon when travelling through the cavity but not outside. (For a readable account of cavity electrodynamics, try ref. 22.) An atom in a long-lived Rydberg state, such as the $63p_{3/2}$ state of rubidium, is well suited to the present problem. In passing through the cavity it couples strongly either to $61d_{3/2}$ or to $61d_{5/2}$ at ~21 GHz as indicated in Fig. 4*a*. These states are currently used in micromaser experiments.

When such an atom is placed in a resonant cavity, it couples much more strongly to the microwave field and in fact decays rapidly from $63p_{3/2}$ (state *a*) to $61d_{5/2}$ (state *b*), for instance, because the mode density in the cavity (see Fig. 4*b*) is much larger than that in free space.

It is possible in principle, and realized in practice, for a Rydberg atom to make the transition $a \rightarrow b$ with unit probability when passing through the cavity, through spontaneous emission of a cavity microwave photon, even when the cavity does not contain photons initially.

Proceeding with the discussion of the micromaser *welcher Weg* detector, we return to Fig. 3 where, immediately preceding the masers, a laser beam is introduced, designed to excite all the atoms from the ground state to the excited state *a*. This can be accomplished by controlling the intensity of the laser beam such that this transition happens with certainty.

In the absence of the laser–cavities system, we now describe the atomic beam, after passing through the double slits, by the state vector

$$\Psi(\mathbf{r}) = \frac{1}{\sqrt{2}}[\psi_1(\mathbf{r}) + \psi_2(\mathbf{r})]|i\rangle \qquad (4)$$

where $\mathbf{r}$ is the centre-of-mass coordinate and i denotes the internal state of the atom. Hence the probability density for particles on the screen at $\mathbf{r} = \mathbf{R}$ is given by the squared modulus of $\Psi(\mathbf{R})$,

$$P(\mathbf{R}) = \tfrac{1}{2}[|\psi_1|^2 + |\psi_2|^2 + (\psi_1^*\psi_2 + \psi_2^*\psi_1)]\langle i|i\rangle \qquad (5)$$

which, of course, agrees with equation (3).

Next consider the situation with the laser turned on and the ultracold (vacuum) micromaser cavities put into the two paths, as in Fig. 3. Before entering the cavities, the laser beam excites the atoms to the long-lived Rydberg state *a*. After passing through the cavities and making the transition $a \rightarrow b$, say, by spontaneous emission of a photon, the state of the correlated atomic beam and maser cavity system is given by

$$\Psi(\mathbf{r}) = \frac{1}{\sqrt{2}}[\psi_1(\mathbf{r})|1_1 0_2\rangle + \psi_2(\mathbf{r})|0_1 1_2\rangle]|b\rangle \qquad (6)$$

where, for example, $1_1 0_2$ denotes the state in which there is one photon in cavity 1 and none in cavity 2. Please note that unlike (4) this $\Psi(\mathbf{r})$ is not a product of two factors, one referring to the atomic and the other to the photonic degrees of freedom. The system and the detector have become entangled by their

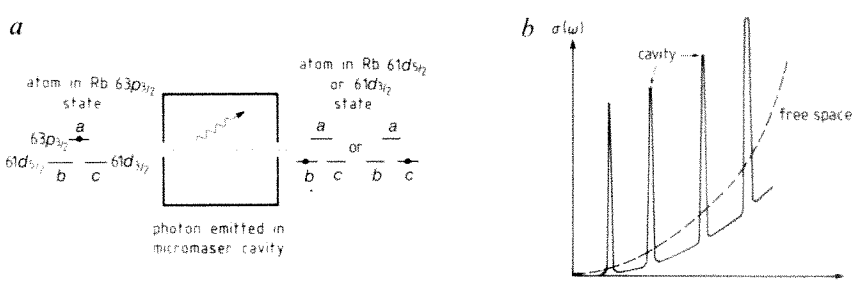

FIG. 4 *a*, Rubidium Rydberg atom in the $63p_{3/2}$ state *a* passes through a micromaser cavity, spontaneously emitting a microwave photon and making a transition to the $61d_{5/2}$ state *b* or the $61d_{3/2}$ state *c*. *b*, Density of photon modes, $\sigma(\omega)$, in free space (dashed curve) and in a micromaser cavity (solid curve), sketched as a function of the frequency ω.

interaction. In contrast to equation (3), the probability density at the screen is now given by

$$P(\mathbf{R}) = \tfrac{1}{2}[|\psi_1|^2 + |\psi_2|^2 + \psi_1^* \psi_2 \langle 1_1 0_2 | 0_1 1_2 \rangle + \psi_2^* \psi_1 \langle 0_1 1_2 | 1_1 0_2 \rangle] \langle b | b \rangle \quad (7)$$

But because $\langle 1_1 0_2 | 0_1 1_2 \rangle$ vanishes, the interference terms disappear here, so that

$$P(\mathbf{R}) = \tfrac{1}{2}[|\psi_1|^2 + |\psi_2|^2] \quad (8)$$

does not show fringes.

The micromasers will serve as *welcher Weg* detectors only if the one extra photon left by the atom changes the photon field in a detectable manner. Thus whether which-path information is available or not depends on the photon states initially prepared in the cavities. One extreme situation has just been discussed: no photons initially, one photon in one of the detectors finally. Clearly, here one can tell through which cavity, and therefore through which slit, the atom came to the screen. The situation is quite different when the cavities contain classical microwave radiation with large (average) numbers of photons, N_1 and N_2, which have spreads given by their square roots. For instance, the change in photon number in cavity 1 is now from $N_1 \pm \sqrt{N_1}$ to $N_1 + 1 \pm \sqrt{N_1}$. This change cannot be detected, because $\sqrt{N_1} \gg 1$, so that there is no which-path information available. (For more details about *welcher Weg* detectors, consult ref. 23.)

In the latter situation (classical radiation in the micromaser cavities), we cannot tell through which slit the atom reached the screen and the interference pattern is just the same as in the absence of the micromaser cavities. In contrast, cavities containing no photons initially store which-path information and therefore the interference pattern is lost. It is changed to the incoherent superposition (8) of one-slit patterns.

We emphasize once more that the micromaser *welcher Weg* detectors are recoil-free; there is no significant change in the spatial wave function of the atoms. It is the correlation of the centre-of-mass wave function to the photon degrees of freedom in the cavities that is responsible for the loss of interference.

In this context, we point out related neutron[24,25] experiments in which radio-frequency fields are employed to change the direction of the magnetic moment and thus the spin state: this does not affect the interference properties of the neutrons. These experiments therefore demonstrate that we can indeed manipulate internal degrees of freedom without changing the centre-of-mass wave function of a quantum system.

BOX 1 The Einstein-Bohr recoiling slit problem

Photons arriving on the (distant) screen of Fig. 1*a* at the location of the first side maximum of the fringe pattern, a distance Δx from the central maximum, possess different momenta $\hbar\mathbf{k}_1$ or $\hbar\mathbf{k}_2$ depending through which slit they reach the screen. The difference Δk_x of the x-component is well approximated by

$$\Delta k_x \approx \frac{2\pi}{\Delta x} \quad (a)$$

The recoil momentum of the plate supporting the slits must therefore be determined with a precision $\Delta p_x \sim \hbar \Delta k_x$, at least, to be able to tell through which slit the photon reached the screen. Thus $\hbar \Delta k_x$ must be distinctly larger than δp_x, the uncertainty in momentum of the slit plate, so that Heisenberg's uncertainty relation

$$\delta p_x \sim \frac{\hbar}{\delta x} \quad (b)$$

where δx is the uncertainty in position of the slit plate, implies

$$\Delta k_x > \frac{1}{\delta x} \quad (c)$$

In view of (a) this tells us that

$$\delta x \geq \Delta x \quad (d)$$

stating that the uncertainty in locating the slits (and therefore the fringes) is larger than the spacing between the fringes. In other words, the fringe pattern is washed out.

Quantum eraser

In the preceding section we have seen that it is the system–detector correlations which account for the dramatic effects of the measuring apparatus on the system of interest. It is no surprise that coherence is destroyed as soon as one has *welcher Weg* information, but here no uncontrollable scattering events (as in Fig. 1*a*; see box) were involved in destroying the interference (wave-like) behaviour.

One then wonders whether it might not be possible to retrieve the coherent interference cross-terms by removing ('erasing') the *welcher Weg* information contained in the detectors. In this sense, we are here considering the quantum eraser problem as discussed sometime ago by M.O.S.[26] (inspired by John Wheeler's suggestion[27] of delayed-choice experiments) and also by others[28–31]. If we erase the *welcher Weg* information in the microwave cavities, will spin coherence be restored? Notice that if we considered the coherence to be lost because of a random scattering or other stochastic perturbations, as studied in ref. 32 and 33, for example, this question would never come up.

In fact, we shall see that interference effects can be restored by manipulating the *welcher Weg* detectors long after the atoms have passed. Edwin Jaynes[29] made some memorable remarks on this problem, which if adapted to the present context would read:

> We have, then, the full EPR [Einstein–Podolsky–Rosen] paradox—and more. By applying or not applying the eraser mechanism before measuring the state of the microwave cavities we can, at will, force the atomic beam into either: (1) a state with a known path, and no possibility of interference effects in any subsequent measurement; (2) a state with both ψ_1 and ψ_2 present with a known relative phase. Interference effects are then not only observable, but predictable. And we can decide which to do after the interaction is over and the atom is far from the cavities, so there can be no thought of any physical influence on the atom's centre-of-mass wavefunction!
>
> From this, it is pretty clear that present quantum theory not only does not use—it does not even dare to mention—the notion of a 'real physical situation'. Defenders of the theory say that this notion is philosophically naive, a throwback to outmoded ways of thinking, and that recognition of this constitutes deep new wisdom about the nature of human knowledge. I say that it constitutes a violent irrationality, that somewhere in this theory the distinction between reality and our knowledge of reality has become lost, and the result has more the character of medieval necromancy than of science. It has been my hope that quantum optics, with its vast new technological capability, might be able to provide the experimental clue that will show us to resolve these contradictions.

In the following we take up Jaynes' challenge, showing how to resolve this "paradox" and suggest further tests of complementarity in quantum mechanics within the framework of modern quantum optics. We present a gedanken experiment involving shutters and ideal photodetectors having unit quantum efficiency. This simple scheme is easy to understand and makes the physics clear, although it may not be possible to realize experimentally. Alternative schemes based on further application of the atomic beam(s)/micromaser combination, which we hope are more experimentally feasible, will be published elsewhere.

Consider now the arrangement of the atomic beam/micromaser system as indicated in Fig. 5*a*. There we see that the atoms pass through the two maser cavity detectors, but now we will imagine that the *welcher Weg* detectors are separated by a shutter–detector combination. So we now have a configuration in which the quantum eraser becomes possible. In particular, consider the cavity system in Fig. 5*a*. There we see two shutters arranged such that radiation will be constrained to remain either in the upper or the lower cavity, when the shutters are closed.

We further imagine that on opening the shutters, light will be allowed to interact with the photodetector wall. In this way the radiation, which is left either in the upper or in the lower cavity, depending upon whether the atom travelled along the upper or lower path, will now be absorbed and the 'memory of passage' (the *welcher Weg* information) could be said to be erased.

Do we now (after erasure) regain interference fringes? The answer is yes, but how can that be? The atoms are now far removed from the micromaser cavities and so "there can be no thought of any physical influence on the atom's centre-of-mass wave function". The answer to this question is given mathematically as follows.

Extending the mathematical description to include the detector, which is initially in its ground state d, we have

$$\Psi(\mathbf{r}) = \frac{1}{\sqrt{2}}[\psi_1(\mathbf{r})|1_1 0_2\rangle + \psi_2(\mathbf{r})|0_1 1_2\rangle]|b\rangle|d\rangle \qquad (9)$$

which replaces equation (6). After absorbing a photon, the detector would be found in the excited state e.

It is now convenient to introduce symmetric, ψ_+, and antisymmetric, ψ_-, atomic states defined as

$$\psi_\pm(\mathbf{r}) = \frac{1}{\sqrt{2}}[\psi_1(\mathbf{r}) \pm \psi_2(\mathbf{r})] \qquad (10)$$

Likewise, we introduce symmetric, $|+\rangle$, and antisymmetric, $|-\rangle$, states of the radiation fields contained in the *welcher Weg* cavities,

$$|\pm\rangle = \frac{1}{\sqrt{2}}[|1_1 0_2\rangle \pm |0_1 1_2\rangle] \qquad (11)$$

In terms of equations (10) and (11), the state (9) of the atom-beam/microwave-cavity/detector system appears as

$$\Psi(\mathbf{r}) = \frac{1}{\sqrt{2}}[\psi_+(\mathbf{r})|+\rangle + \psi_-(\mathbf{r})|-\rangle]|b\rangle|d\rangle \qquad (12)$$

We now consider the interaction between the radiation field existing in the cavity and the detector. As mentioned earlier, we envisage the detector to consist of an atom with a lower state d and an excited state e. The interaction hamiltonian between field and detector depends on symmetric combinations of the field variables, so that only the symmetric state $|+\rangle$ will couple to the fields.

We then find that the action of the detector (eraser) system produces the state

$$\Psi(\mathbf{r}) = \frac{1}{\sqrt{2}}[\psi_+(\mathbf{r})|0_1 0_2\rangle|e\rangle + \psi_-(\mathbf{r})|-\rangle|d\rangle]|b\rangle \qquad (13)$$

That is, the symmetric interaction couples only to the symmetric radiation state $|+\rangle$; the antisymmetric state $|-\rangle$ remains unchanged.

Now, the atomic probability density at the screen goes as

$$P(\mathbf{R}) = \tfrac{1}{2}[\psi_+^*(\mathbf{R})\psi_+(\mathbf{R}) + \psi_-^*(\mathbf{R})\psi_-(\mathbf{R})]$$
$$= \tfrac{1}{2}[\psi_1^*(\mathbf{R})\psi_1(\mathbf{R}) + \psi_2^*(\mathbf{R})\psi_2(\mathbf{R})] \qquad (14)$$

and does not show any interference fringes as long as the final state of the detector is unknown. But if one asks what is the probability density $P_e(\mathbf{R})$ for finding both the detector excited and the atom at $\mathbf{R}$ on the screen, the answer is

$$P_e(\mathbf{R}) = |\psi_+(\mathbf{R})|^2$$
$$= \tfrac{1}{2}[|\psi_1(\mathbf{R})|^2 + |\psi_2(\mathbf{R})|^2] + \mathrm{Re}[\psi_1^*(\mathbf{R})\psi_2(\mathbf{R})] \qquad (15)$$

which exhibits the same fringes as equation (3), indicated as a solid line in Fig. 5*b*. In contrast, the probability density $P_d(\mathbf{R})$ for finding both the detector deexcited and the atom at $\mathbf{R}$ on the screen is

$$P_d(\mathbf{R}) = |\psi_-(\mathbf{R})|^2$$
$$= \tfrac{1}{2}[|\psi_1(\mathbf{R})|^2 + |\psi_2(\mathbf{R})|^2] - \mathrm{Re}[\psi_1^*(\mathbf{R})\psi_2(\mathbf{R})] \qquad (16)$$

giving rise to the antifringes indicated by the broken line in Fig. 5*b*. If the eraser photon signal is disregarded, one obtains the superposition (14), equal to half the sum of P_e and P_d, which is fringeless, and, of course, identical with (8).

Here is the physical interpretation of this calculation. After an atom has run the gauntlet from the oven to the screen, passing through micromasers and leaving its tell-tale photon, we record an event somewhere on the screen. Then we return to the *welcher Weg* micromasers, open the shutters and allow the absorption of the microwave photon. When we observe a photocount in the detector we know that erasure has been completed. In this event the atom is counted as a 'yes'-atom.

Then we wait for another atom to pass through the system from oven to screen. Again we record an event on the screen and then turn to the micromaser cavities. This time suppose that, upon opening the shutter, we observe no photocount in the quantum eraser detector. This will be the case half of the time, as explained above. Now we count the atom as a 'no'-atom.

We repeat the above sequence many times. Eventually, the 'yes'-atoms will build up the solid-line fringes in Fig. 5*b*, and the 'no'-atoms produce the broken-line antifringes. Finally we note that the fringes and antifringes will cancel if we do not correlate them to the state of the eraser-detector. In this way we have resolved the 'Jaynes paradox'.

Having presented the physics of quantum erasure we now turn to an experimentally more realizable scheme which has much in common with the quantum eraser idea. We consider, as in Fig. 6, the asymmetric situation in which cavity 1 is tuned to the transition $a \rightarrow b$ $(63p_{3/2} \rightarrow 61d_{3/2})$, and cavity 2 is tuned to the transition $a \rightarrow c$ $(63p_{3/2} \rightarrow 61d_{5/2})$.

Even if the cavities contain classical microwave radiation, as we shall assume in the sequel, and therefore do not store which-path information, the screen will not show interference fringes because the internal atomic states b and c are orthogonal. This is analogous to the disappearance of the interference terms in equation (7), except that now the atoms themselves carry the *welcher Weg* information.

The latter circumstance again invites the question: could one not induce the transitions $b \rightarrow c$ in the atoms that traversed

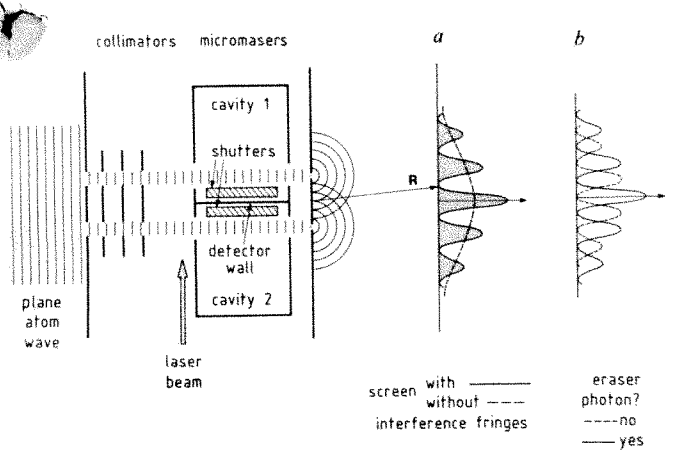

FIG. 5 *a*, Quantum erasure configuration in which electro-optic shutters separate microwave photons in two cavities from the thin-film semiconductor (detector wall) which absorbs microwave photons and acts as a photodetector. *b*, Density of particles on the screen depending upon whether a photocount is observed in the detector wall ('yes') or not ('no'), demonstrating that correlations between the event on the screen and the eraser photocount are necessary to retrieve the interference pattern.

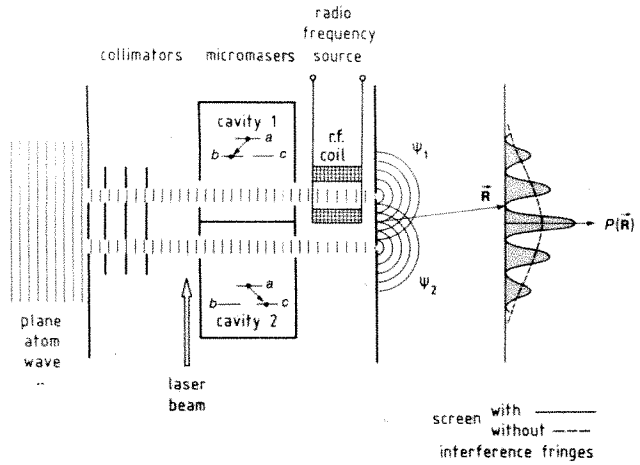

FIG. 6 Asymmetric set-up in which cavity 1 induces the transition $a \rightarrow b$ and cavity 2 induces $a \rightarrow c$. Which-path information is erased by the radio frequency in the coil where $b \rightarrow c$ happens.

cavity 1, so that the which-path information is erased, and thereby make the interference pattern reappear? The answer is affirmative. The actual experimental realization, however, is a delicate matter, because one must exert careful control on the phases of the various classical radiation fields. To appreciate what is involved, suppose that between cavity 1 and the slit plate there is a coil that can be fed with radio frequency of ~50 MHz with the right strength to ensure the transition $b \rightarrow c$, as depicted in Fig. 6. In the interference region the state of the atom is essentially

$$\Psi(\mathbf{r}) = \frac{1}{\sqrt{2}}[\psi_1(\mathbf{r}) + e^{i\beta}\psi_2(\mathbf{r})]|c\rangle \qquad (17)$$

where the relative phase angle β is determined by the phases of the microwave fields in the two maser cavities and the radio-wave field in the coil. As these fields have different frequencies, β really refers to a certain instant, the moment, say, when the atom is excited to state a by the laser beam. The probability density at the screen

$$P(\mathbf{R}) = |\Psi(\mathbf{R})|^2 = \tfrac{1}{2}(|\psi_1|^2 + |\psi_2|^2) + \mathrm{Re}\,(\psi_1^* e^{i\beta}\psi_2) \qquad (18)$$

now exhibits an interference term that depends on β very sensitively. If, therefore, the value of β varies from atom to atom, the interference pattern will not build up. This illustrates quite well the omnipresent phenomenon of coherence loss caused by random phases. Consequently, one must ensure that the phase angle β is the same for all atoms to make the interference fringes reappear. In the set-up of Fig. 6 this can be achieved by adjusting the phase of the radiofrequency radiation in the coil to the phases that the microwave fields in the cavities have at the moment when the laser excites the atom. An additional bonus is the possibility of varying the chosen value of β, which enables one to shift the interference pattern on the screen. In summary, the control over the phase angle β represents a switch with which the experimenter can turn the interference fringes on and off, or relocate them.

Thus it would seem that the way is open for experiments of the *welcher Weg*/quantum-eraser type. No doubt they will be difficult, but as we gain more experience with these 'amazing masers', experiments along these lines will one day be realized, and *welcher-Weg*-type experiments are now under way at the Max-Planck-Institut in Garching. □

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Male development of chromosomally female mice transgenic for *Sry*

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The initiation of male development in mammals requires one or more genes on the Y chromosome. A recently isolated gene, termed *SRY* in humans and *Sry* in mouse, has many of the genetic and biological properties expected of a Y-located testis-determining gene. It is now shown that *Sry* on a 14-kilobase genomic DNA fragment is sufficient to induce testis differentiation and subsequent male development when introduced into chromosomally female mouse embryos.

THE processes of morphogenesis and cellular differentiation are dependent on the activity of sets of genes in complex interacting networks or pathways. Within these pathways, specific genes may be rate-limiting or act as a switch, such that their activity seems to cause a developmental event. One case where this occurs is in sex determination, where the pathways of gene activity that lead to male or female development must include a switch mechanism to determine which pathway is chosen[1,2]. In mammals, the Y chromosome acts as a dominant male determinant[3-5] as it carries a gene or genes with a critical role in the male pathway. The central event in mammalian sex determination is the differentiation of testes rather than ovaries from the indifferent gonad (genital ridge)[6,7]. All other differences between the sexes in eutherian mammals are secondary effects due to hormones or factors produced by the gonads. For this reason sex determination is equivalent to testis determination, and the Y-chromosomal gene(s) responsible has been named *Tdy* (testis-determining gene on the Y) in mice, and *TDF* (testis-determining factor) in humans.

By exploiting detailed maps of the sex-determining region of the human Y chromosome, we cloned the gene *SRY* and its mouse equivalent *Sry*[8,9]. These genes are located in the smallest regions of the human and mouse *Y* chromosomes known to be male-determining, and conserved homologues have been found on the Y chromosomes of all other eutherian mammals tested. Furthermore, *Sry* is deleted from a mouse Y chromosome with a mutant *Tdy*[9-11]. As might be predicted for a regulatory gene, *SRY*/*Sry* encodes a protein containing a DNA-binding motif[8,9]. *Sry* also shows a pattern of expression in the mouse entirely consistent with a role in testis determination, being expressed for a short period from about 10.5–12 days *post coitum* (d.p.c.) just before overt testis differentiation, specifically in the somatic cells of the genital ridge[12].

Direct evidence that *SRY*/*Sry* has a role in testis formation was obtained from the analysis of the genomes of XY females with gonadal dysgenesis. In two cases, sex-reversed XY daughters were found to have mutations in *SRY* that were not present in their fathers[13,14]. This correlation between *de novo* mutation in *SRY* and sex reversal implies that *SRY*/*Sry* is required for normal testis formation, but the experiments do not address whether it alone is equivalent to the genetically defined factor *TDF*/*Tdy*.

The best way to test the function of *SRY*/*Sry* is to introduce it into XX embryos, and to see if they develop as males. The pattern of *Sry* expression during fetal gonad development in the mouse suggests that precise regulation of the gene may be critical for its action[12]. We therefore introduced murine *Sry* or human *SRY* as part of a genomic fragment, in the expectation that this would provide the correct regulatory sequences. Although the human gene does not seem to function in mice, mouse *Sry* in a 14-kilobase (kb) genomic fragment gives rise to normal testis development in chromosomally female transgenic mice, as can be seen at both embryonic and adult stages.

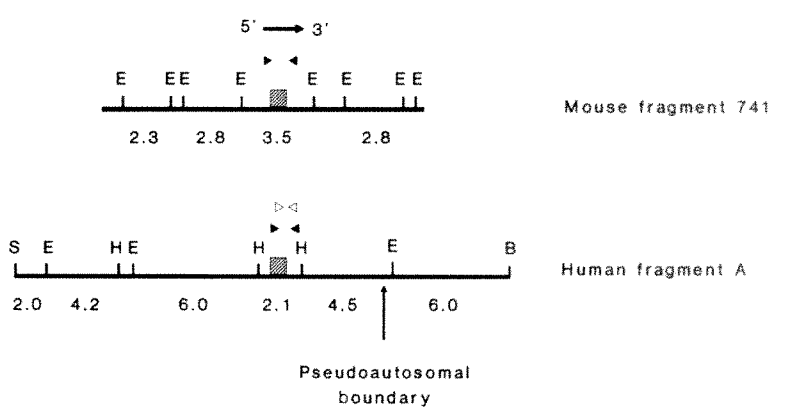

FIG. 1 Genomic DNA frragments used for microinjection. Restriction maps of mouse *Sry* fragment 741 (ref. 9) and human *SRY* fragment A (isolated from the cosmid cAMF; ref. 29). Restriction-endonuclease sites: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII and S, *Sal*I. Fragment sizes are indicated in kb. The conserved *Sry*/*SRY* open reading frame is indicated by a shaded box. The direction of the open reading frame is shown above the two clones. The position of the human pseudoautosomal boundary is indicated, the pseudoautosomal region being to the right of this point. The positions of oligonucleotide primers used for PCR analysis (described in the legends to Figs 3 and 5) are indicated by triangles.
METHODS. *SRY*- or *Sry*-containing fragments were released from cosmid or phage vectors by digestion with appropriate restriction enzymes and then isolated by agarose gel electrophoresis and further purified by one of three methods: (1) Geneclean (Bio101), according to manufacturer's instructions; (2) phenol extraction, Sephadex G50 column chromatography and ethanol precipitation; (3) Geneclean followed by elutip (Schleicher & Schuell) and ethanol precipitation. Transgenic mice were produced essentially as described by Hogan *et al.*[38]. In brief, three- to five-week-old (CBA × C57BL/10) F_1 females were superovulated and mated to F_1 stud males. The next day, fertilized eggs were collected from oviducts of females showing vaginal plugs. Pronuclei were microinjected with 1–2 pl of DNA at a concentration of approximately 2 $\mu g\ ml^{-1}$. The eggs were cultured overnight in M16 or T6 medium, and 2-cell embryos implanted by oviduct transfer into day-of-plug pseudopregnant recipients.

Sex reversal of transgenic mouse embryos

Fertilized eggs were microinjected with *Sry* gene sequences and transferred to pseudopregnant recipients, and some of the resulting embryos were analysed 14 days later rather than allowing them all to develop to term. The first visible sign of testis development from the genital ridge is the formation of testis cords at about 12.5 dpc in the mouse. This is due to the differentiation of Sertoli cells and their alignment into epithelial structures surrounding the germ cells[15]. Cord formation confers a characteristic striped appearance to the developing testis, distinguishing it from the fetal ovary. Other morphological changes characteristic of the testis are its rapid growth and prominent vasculature. Phenotypic sex can be rapidly assayed by examining fetuses about 14 days after oviduct transfer, when testis-cord formation is obvious even with partial sex reversal[16,17]. An indication of chromosomal sex was obtained by staining for sex chromatin in amnion cells[18]. Where necessary, this was confirmed by Southern blot analysis or the polymerase chain reaction (PCR) using a DNA probe or oligonucleotide primers derived from the Y-linked gene *Zfy-1* (refs 19–21).

A 14-kb fragment derived from a phage clone L741 (ref. 9), containing about 8 kb of sequence upstream and 5 kb downstream, of the putative DNA-binding domain of *Sry* (Fig. 1), was purified from vector sequences and used to generate transgenic mice. After injection with this fragment (subsequently referred to as f741), 158 embryos were obtained. Most of these were XY males or XX females, in roughly equal porportion (Table 1). However, in two cases testes were seen in embryos whose sex chromatin indicated an XX rather than an XY sex chromosome constitution. Southern-blot analysis showed that

TABLE 1 Analysis of mouse fragment 741 transgenic embryos

No. of embryos	Sex chromatin	*Sry*	*Zfy*	Deduced karyotype	Transgenic	Phenotypic sex
63	+	−	27-/40 ND	XX	−	♀
27	−	+	+	XY	ND	♂
58	−	ND	ND	XY	ND	♂
2	−	−	−	XO	−	♀
6	**+**	**+**	**−**	**XX**	**+***	**♀**
2	**+**	**+**	**−**	**XX**	**+**	**♂**

Injected embryos were examined 14 days after transfer. Embryos were analysed in the following way: chromosomal sex (XX or XY/XO) was determined by staining for sex chromatin in amnion cells[18]. Transgenesis was assayed either by Southern blot or PCR detection of *Sry*, and the presence or absence of a Y chromosome judged from similar assays for *Zfy* gene sequences. Phenotypic sex was determined by scoring for testis or ovary development. The frequency of XO progeny was consistent with previous studies[35]. Asterisk indicates that in four cases of XX transgenesis, comparison of the *Sry* signal to that of a control male indicated mosaicism for the transgene. The bold type highlights the transgenic embryos obtained. ND, not determined. Genomic DNA for Southern analysis was prepared from limbs essentially as described[36]. For PCR analysis proteinase K digestion was performed in 1 mM EDTA, the DNA was extracted once with phenol/chloroform and a small aliquot added directly to the PCR reaction mixture. Southern analysis of *Eco*RI-digested DNA was performed as described by Maniatis *et al.*[37]. For *Sry*, blots were probed with clone 422.04 (see ref. 9) which contains the *Sry* conserved motif. For the *Zfy* genes, a 1.9-kb *Hin*dIII genomic fragment containing the region encoding the *Zfy-1* zinc-finger domain[11,21] was used as a probe. PCR analysis was performed as described in Fig. 3 legend.

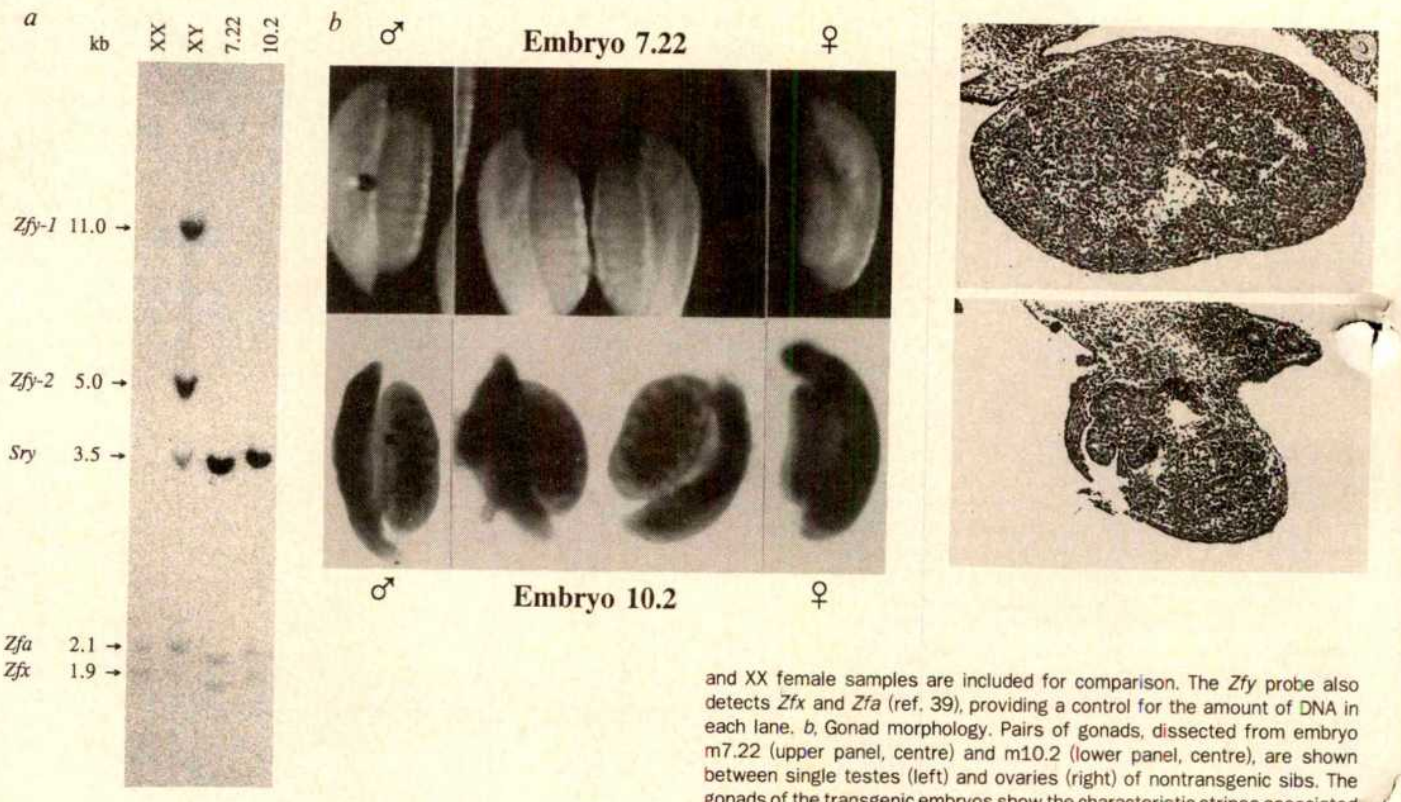

FIG. 2 Analysis of sex-reversed transgenic embryos. a, Southern-blot analysis of DNA from two phenotypic male embryos m7.22 and m10.2. Lack of hydridization to a probe recognizing *Zfy-1* and *Zfy-2* shows the absence of a Y chromosome, whereas the intensity of hybridization to the *Sry* probe demonstrates that they carry multiple copies of the transgene. XY male and XX female samples are included for comparison. The *Zfy* probe also detects *Zfx* and *Zfa* (ref. 39), providing a control for the amount of DNA in each lane. *b*, Gonad morphology. Pairs of gonads, dissected from embryo m7.22 (upper panel, centre) and m10.2 (lower panel, centre), are shown between single testes (left) and ovaries (right) of nontransgenic sibs. The gonads of the transgenic embryos show the characteristic stripes associated with testis-cord formation. *c*, Histology of m7.22 (upper panel) and m10.2 (lower panel) testis sections. The apparent difference in size is due to plane of section. Cord morphology was similar to that of littermates (not shown). METHODS. Southern-blot analysis and probes are described in Table 1 legend. Gonads were photographed whole in PBS, then fixed in 4% paraformaldehyde, dehydrated in ethanol and embedded in paraffin. Sections (7 μm) were stained in haematoxylin and eosin.

both of these males lacked *Zfy* sequences and were transgenic, with many copies of *Sry* (Fig. 2*a*). Histological examination showed that their testis-cord formation was normal and that their gonads were indistinguishable from testes of normal XY sib embryos (Fig. 2*b*, *c*).

From these experiments we conclude that a 14-kb genomic fragment carrying *Sry* sequences is sufficient to initiate testis development in mice.

To determine the frequency with which f741 gives sex reversal, all the embryos scored as females were examined for the presence of *Sry* sequences by PCR. Two were unequivocally identified as transgenic for *Sry*, and four more were probably mosaics possessing the transgene in a low proportion of cells, as only weak signals for the presence of *Sry* sequences were detected (Table 1). We attribute the finding that not all XX transgenics show sex reversal to such mosaicism or to position effects on *Sry* expression (see below).

Normal adult male phenotype

To test the adult phenotype of *Sry* transgenic mice some of the embryos injected with f741 were allowed to develop to term. A total of 93 animals were born (49 males and 44 females). Southern blotting showed that five of these were transgenic. Two were XY males that did not transmit the transgene and so were uninformative with respect to sex reversal.

One of the transgenics, m33.13, had no Y chromosome as determined by PCR analysis (Fig. 3*a*) but was externally male (Fig. 3*b*). He was similar in size and weight to his normal XY male littermates. At about six weeks *post partum*, m33.13 was caged with females (a maximum of two per night). His copulatory behaviour was normal, mating four times in six days.

The presence of two X chromosomes in a male mouse always results in sterility, as germ cells are prevented from progressing beyond prospermatogonia. This phenomenon has been documented in XX Sxr and XX Sxr′ mice which are male owing to the presence of Y-derived sequences including *Tdy* on one of their X chromosomes[22-24]. It was therefore nor surprising that the sex-reversed transgenic mouse m33.13 was also sterile. None of the four females with which he mated became pregnant. In three cases the vaginal plugs were examined for the presence of sperm, but none was found (data not shown). The only difference between m33.13 and a normal XY sibling was in the size of the testes: m33.13 had a testis weight of 17 mg (in the range expected for an XX Sxr′ male), as opposed to 76 mg for an XY control littermate. Histological examination of sections of the testes revealed the presence of tubules, with clearly defined and apparently normal populations of Leydig cells, peritubular myoid cells and Sertoli cells, but no cells undergoing spermatogenesis (Fig. 3*c*).

Internal examination of m33.13 revealed a normal male reproductive tract with no signs of hermaphroditism. This indicates that Sertoli and Leydig cells must have functioned normally in producing anti-Müllerian hormone (AMH) and testosterone, respectively. AMH is required for the elimination of the female Müllerian duct system (oviducts, uterus and upper vagina) and testosterone for the development of the Wolffian duct derivatives (vas deferens and accessory glands such as the seminal vesicles) and male secondary sexual characteristics[15,25].

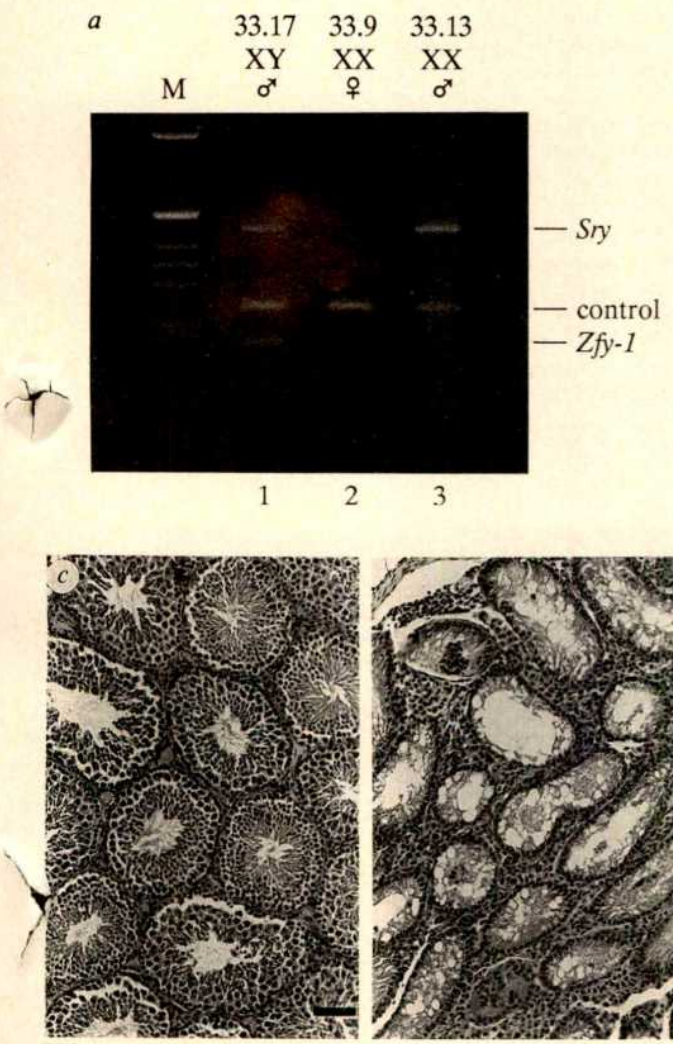

FIG. 3 Analysis of adult sex-reversed transgenic mouse m33.13. *a*, PCR analysis of genomic DNA from m33.13 (lane 3), showing *Sry* and control (myogenin) bands. No band corresponding to *Zfy-1* was seen, demonstrating the lack of a Y chromosome; this result was confirmed by Southern blotting using Y-chromosome probes Y353B (ref. 40) and Sx1 (ref. 41) (not shown). Normal XX female and XY male littermates (33.9, lane 2 and 33.17, lane 1) are shown for comparison. M, marker bands (1,018, 510, 396, 344, 298, 220, 201, 154 and 134 base pairs). *b*, External genitalia of mice 33.17 (left) and 33.13 (right). *c*, Histology of testis sections from mice 33.17 (left) and 33.13 (right). Bar, 90 μm.

METHODS. For PCR analysis, 0.1 μg genomic DNA was added to a 50-μl reaction mixture containing 1.5 mM each dNTP, 50 mM Tris–HCl, pH 9, 15 mM ammonium sulphate, 7 mM $MgCl_2$, 0.05% Nonidet P-40, 0.5 U *Taq* polymerase (Anglian Biotec) and 500 ng each oligonucleotide primer. Amplification consisted of 30 cycles of 94 °C for 5 s, 65 °C for 30 s and 72 °C for 30 s in a Techne PHC-2 thermocycler. An 8-μl aliquot was electrophoresed on a 2% agarose–TBE gel. Primers for *Sry* were (5′–3′) TCATGAGACTGCCAACCACAG and CATGACCACCACCACCACCAA (indicated as triangles in Fig. 1) and for *Zfy-1*, CCTATTGCATGGACTGCAGCTTATG and GACTAGACATGTCTTAACATCTGTCC; myogenin primers corresponded to nucleotides 656–675 and 882–901 of the rat complementary DNA sequence[42]. PCR products were 441, 180 and 245 bp, respectively. Testes were processed for histological examination as described in Fig. 2 legend.

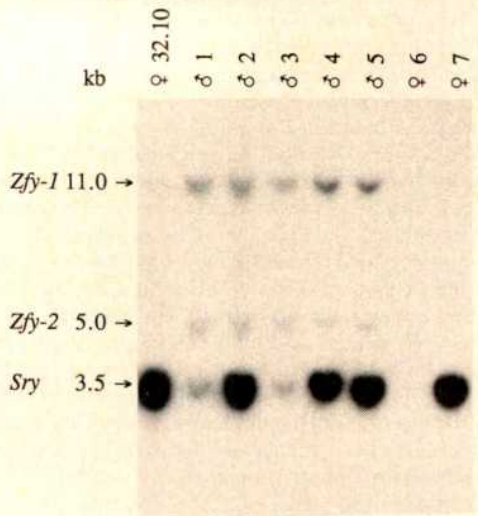

FIG. 4 Southern-blot analysis of offspring derived from transgenic female m32.10. As described in Fig. 2*a*, lack of hybridization to a probe recognizing *Zfy-1* and *Zfy-2* indicates the absence of a Y chromosome. Founder female m32.10 hybridizes intensely to the probe recognizing *Sry* demonstrating the presence of multiple copies of the transgene. The same band pattern is shown by offspring number 7. Examination of the external genitalia of this animal revealed a normal female phenotype. DNA from the male offspring 1–5 hybridizes to both *Zfy* and *Sry* probes. Three of these have multiple copies of *Sry* and are therefore transgenic.
METHODS. Mouse m32.10 was mated with an F_1 (CBA × C57BL/10) male and biopsies of the resulting offspring's tails made at 3 weeks. Genomic DNA preparation and Southern-blot analysis were as described in Table 1 legend.

A further two XX transgenics, m32.10 and m33.2, showed an external female phenotype, yet both carried many copies of *Sry*. These mice have produced offspring and so have functional reproductive tracts and ovaries. They also provide further evidence, along with the transgenic XX female fetuses, that f 741 does not always cause sex reversal. Although there could be subtle rearrangements of the *Sry* gene making it non-functional, the possibility of this occurring in all these cases is remote. There are two more probable explanations. First, these females could be mosaic for the transgene, with only a small proportion of the cells making up the somatic portion of the genital ridge carrying functional *Sry* gene copies. Analysis of XX↔XY chimaeras suggests that females or hermaphrodites develop if less than about 30% of cells are XY[17,26,27]. Secondly, the expression of the transgene could be affected by the position at which it integrates. Except for a few cases where locus-controlling regions are present, expression of transgenes almost always depends on their chromosomal location[28]. These two alternatives can be examined by breeding from the adult XX transgenic females. Mouse m33.2 has not yet produced transgenic offspring. However, m32.10 has transmitted the transgene to female offspring (Fig. 4), suggesting that it is not mosaic.

Human *SRY* does not function in mice

Mouse *Sry* and human *SRY* have a highly similar nucleotide sequence in the region encoding the putative DNA-binding domain[9]. However, the two domains differ in 23 of their 79 amino-acid residues, with only two of the differences being conservative. The specificity of interaction of *SRY*/*Sry* with other genes in the sex determination pathway presumably depends on the structure of this domain. That only a single amino-acid substitution can lead to failure of testicular development in an XY individual[13] suggests that even a subtle alteration in the *SRY* protein could disrupt its action. In addition, the nucleotide sequences of the mouse and human genes diverge strikingly outside the region encoding the putative DNA-binding domain[9]. We therefore tested whether the human *SRY* gene is as effective as its murine counterpart in causing sex reversal in mice.

The DNA used for pronuclear injection was a 25-kb *Bam*HI–*Sal*I fragment representing human Y chromosomal DNA around *SRY*, isolated from a cosmid clone cAMF (ref. 29). This fragment includes roughly 12.5 kb of Y-unique sequence upstream, and 5 kb downstream, of the *SRY* conserved domain. The remaining 6 kb represents sequences from the pseudoautosomal region, which is common to the X and Y chromosomes (Fig. 1).

The ability of human *SRY* to cause sex reversal was assessed in animals representing three independent integrations of the transgene. Two of these were lines derived from XY founder

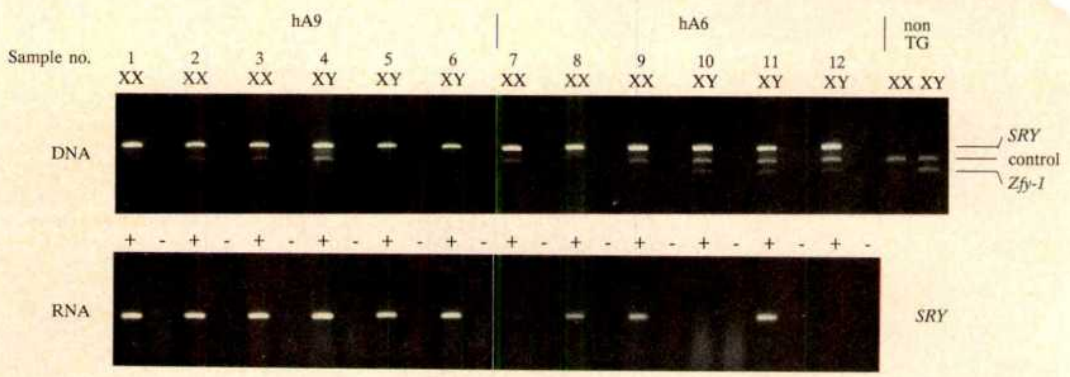

FIG. 5 Expression of human *SRY* in transgenic mouse embryos. Three male and three female embryos from each of two lines (hA9 and hA6) transgenic for *SRY* were analysed at 11.5 d.p.c. PCR analysis of genomic DNA (upper panel) showed that all embryos (lanes 1–12) were transgenic for *SRY*. Samples 4–6 and 10–12 showed bands corresponding to *Zfy-1*, confirming that they were XY, unlike samples 1–3 and 7–9 which were XX. Non-transgenic controls (non TG) are shown on the right. Oligonucleotide primers for myogenin were included in each analysis as a control. Below each lane is shown a PCR analysis of reverse-transcribed RNA extracted from the urogenital ridges of the same embryos (lower panel). Reverse transcription was performed on each RNA sample with (+) and without (−) reverse transcriptase, to demonstrate that the observed bands were due to the presence of *SRY* transcripts and not contaminating DNA. Bands corresponding to *SRY* expression were seen in samples 1–9 and 11.
METHODS. RNA was extracted from pairs of urogenital ridges and reverse-transcribed in small-scale reactions as described[21], using half the yield from each pair of ridges in the + and − reverse-transcriptase reactions. Genomic DNA or reverse-transcription products were added to PCR reactions and amplified as described in Fig. 3 legend. For DNA analysis, *SRY* primers used were (5′–3′) GATCAGCAAGCAGCTGGGATACCAGTG and CTGTAGCGGTCCCGTTGCTGCGGTG (336-bp product, solid triangles in Fig. 1); for RNA analysis, CAGGAGGCACAGAAATTACAGGGCTGC and ACAGTCATCCCTGTACAACCTGTTGTCC (174-bp product; open triangles in Fig. 1) were used.

transgenics, hA6 and hA9. These founders transmitted *SRY* to about half of their offspring: 8 out of 17 hA6 and 13 out of 36 hA9 embryos assayed were transgenic. We analysed progeny of these lines at 14.5 d.p.c., and found no evidence of testis-cord formation in XX transgenic fetuses, demonstrating that neither of the integrations in these lines could cause sex reversal. A third integration was represented by a single XX transgenic founder embryo; this too was phenotypically female.

Having transgenic lines that transmit human *SRY* to their offspring allowed us to examine expression of the transgene in developing gonads, and in the only other known site of *Sry* expression, the adult testis[8,12]. Genital ridges were dissected from hA6 and hA9 fetuses at 11.5–12 d.p.c., when mouse *Sry* is expressed[12]. PCR analysis of RNA extracted from these samples shows that *SRY* transcripts were present in transgenic XX fetuses that were not sex-reversed (Fig. 5). Curiously, not all hA6 XY fetuses expressed the transgene (Fig. 5); this could be due to variation in developmental stage. The level of SRY expression in the genital ridges was estimated to be several times that of the endogenous *Sry* gene, and was greater than that seen in transgenic XY adult testis material (not shown).

Clearly a lack of transcription in the genital ridge cannot account for the failure of *SRY* to give sex reversal in mice. It is formally possible that the *SRY* mRNA is not correctly processed or translated. Alternatively, the protein product could be unstable in mouse cells. However, it is more likely that differences in sequence result in the human *SRY* protein failing to interact with other regulatory proteins or target genes in mouse cells. This hypothesis could be tested by exchanging the human and mouse open reading frames.

Discussion

The experiments described here demonstrate that a 14-kb mouse genomic DNA fragment containing *Sry* is sufficient to direct the formation of testes in XX transgenic embryos and subsequently to give rise to full phenotypic sex reversal in an XX transgenic adult. Complete sequencing of f741, as well as cross-hybridization experiments to human DNA, have failed to detect any gene sequences other than *Sry* (D. Jackson and A. Sinclair, unpublished results). Although previous data provided compelling evidence to implicate *Sry* in the process of testis determination, this study suggests that *Sry* is the only Y-linked gene required to give rise to male development, and we propose that *Sry* is *Tdy*.

The ability of a 14-kb fragment containing *Sry* to cause sex reversal suggests that this fragment contains the entire *Sry* gene, including all the regulatory elements required for appropriate embryonic expression. The transgenic system can be used to localize further these regulatory elements within the 14-kb fragment by microinjecting progressively smaller constructs. The only other site of *Sry* expression is in adult testis, probably in the germ-cell component[12]. It will be interesting to see if the 14-kb fragment f741 also contains the regulatory information required for the switch from expression in the somatic part of the embryonic gonad to expression in adult testis associated with germ cells. Our finding that the human *SRY* gene is expressed at both stages in transgenic mice is consistent with the appropriate regulatory sequences being present within the 25 kb of DNA containing the gene. It also indicates that the mechanisms regulating *Sry*/*SRY* expression have been conserved between humans and mice.

Although we have shown that *Sry* alone can promote testicular development in the absence of other Y-linked genes, sex reversal does not always occur. The most likely explanation for this is that the *Sry* transgene is sensitive to position effects. This variability may mirror the situation in human XX individuals carrying small portions of the Y chromosome including *SRY*. Palmer *et al.*[30] described four such cases, each of whom had inherited about 35 kb of Y-unique sequences on their paternal X chromosome, two of whom were only partially sex-reversed. Jäger *et al.*[31] also describe an XX hermaphrodite with a similar small portion of the Y. In these cases *SRY* may be affected not only by adjacent DNA sequences in its new chromosomal location, but also by the spread of X-inactivation. There are several precedents for the latter in the mouse; for example females or hermaphrodites frequently develop instead of males when the Sxr fragment is present only on the inactive X chromosome[32]. There is of course no reason to believe that X-inactivation is involved in expression of the transgene considered here. Nevertheless, when additional transgenic mice are analysed, we would expect to find instances of partial sex reversal due to position effects, where the level of *Sry* expression is close to a critical threshold. It will be important to understand what this threshold means in the process of testis determination.

Sry acts over a short time to initiate testis development. It must do this through interaction with other genes, some of which will be involved in the regulation of *Sry*, others of which will be downstream targets of *Sry*. These other genes must map elsewhere in the genome, because *Sry* is shown here to be the only Y-linked gene required to bring about male development in mice. Mutations in some of these genes could explain cases of male development in XX individuals lacking *SRY*[30], and XY females where *SRY* is intact[13,14,33,34]. Using molecular genetic techniques to work stepwise from *Sry* it should now be possible to identify these other genes. □

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Ha-Ras augments c-Jun activity and stimulates phosphorylation of its activation domain

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Ha-Ras augments c-Jun-mediated transactivation by potentiating the activity of the c-Jun activation domain. Ha-Ras also causes a corresponding increase in phosphorylation of specific sites in that part of the c-Jun protein. A Ha-Ras-induced protein kinase cascade resulting in hyperphosphorylation of the c-Jun activation domain could explain how these oncoproteins cooperate to transform rat embryo fibroblasts.

PROTO-ONCOGENE c-*jun* encodes a nuclear protein capable of transforming either chicken cells[1,2], immortalized rat fibroblasts[3] or primary rat embryo fibroblasts (REF) in cooperation with activated Ha-Ras (ref. 3). The c-Jun protein is a major component of the transcription factor AP-1 (refs 4, 5), originally shown to mediate phorbol ester tumour promoter (12-O-tetradecanoylphorbol-13-acetate; TPA)-induced expression of responsive genes through the TPA-response element (TRE)[6,7], a specific site in their promoters. Other AP-1 components belong either to the Jun (JunB, JunD) or Fos (c-Fos, FosB, Fra1) families. The Jun proteins form homo- and heterodimers which bind the TRE, but the Fos proteins are active only as heterodimers with any of the Jun proteins[1,8]. Among the Jun proteins, only c-Jun activates transcription of target genes containing a single TRE in the absence of Fos[9-11].

The *jun* and *fos* genes are induced by external stimuli such as TPA and growth factors[12-16]. Induction of c-*jun* by TPA is thought to result from a positive autoregulatory mechanism[16], and to involve dephosphorylation of preexisting c-Jun at inhibitory phosphorylation sites next to its DNA-binding domain[17]. AP-1 activity is also induced by neoplastic transformation[18,19] and transient expression of transforming oncogenes such as activated Ha-*ras*[20,21]. Stimulation of AP-1 activity by transforming oncogenes is not fully understood but is probably important in the neoplastic process because AP-1 is the major transcription factor whose activity is augmented by cellular transforming oncogenes[22], possibly accounting for altered patterns of gene expression in malignant cells. It was suggested that the effect of Ha-Ras on AP-1 activity was through induction of c-*fos* and c-*jun*[22,23]. But induction of c-*fos* by Ha-Ras is transient[24] and there is no evidence for elevated c-*fos* expression in most transformed cells. The findings that Ha-*ras* cooperates not only with c-*jun*[3] but also with TPA[25], an inducer of c-*jun*, and that cells resistant to transformation by v-*fos* are also resistant to Ha-*ras*[26] and c-*jun*[27], led us to examine the effect of activated Ha-Ras on c-Jun activity. We found that expression of activated Ha-Ras caused a marked increase in transcriptional activation by c-Jun in various cell types, including REF, independently of c-*fos* induction. This stimulation results from the increased potency of the c-Jun N-terminal activation domain and correlates with its increased phosphorylation.

Ha-Ras stimulates transactivation by c-Jun

Stimulation of c-Jun activity by Ha-Ras could account for the oncogenic cooperation between c-*jun* or TPA and Ha-*Ras*[3,25]. A CAT (chloramphenicol acetyltransferase) reporter gene controlled by a TRE-containing collagenase promoter (−73Col-CAT)[5] was used to examine transactivation by c-Jun in REF cells. Ha-Ras protein was provided by pZIPneoRas which encodes an activated form (leu61) of human Ha-Ras[28]. Alone, pZIPneo-Ras stimulated CAT activity two- to threefold (Fig. 1). Although of smaller magnitude, this effect is consistent with stimulation of AP-1 activity of Ha-Ras in established cell lines[20,21]. Ha-Ras also increased transactivation by c-Jun three- to fourfold. Transactivation of v-Jun was also stimulated but to

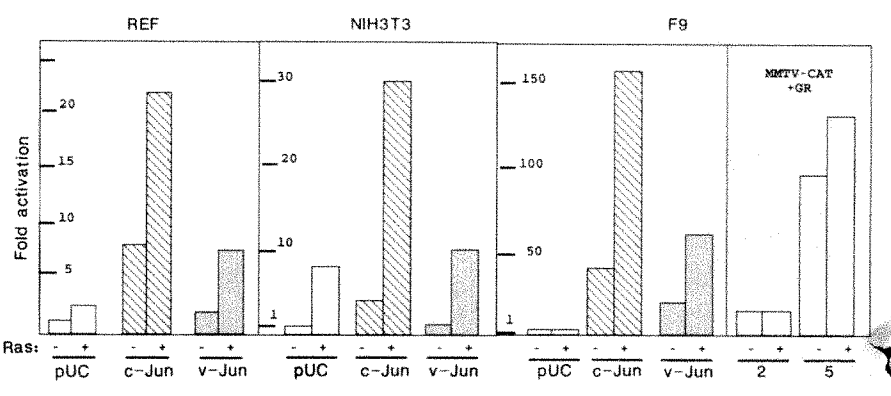


FIG. 1 Ha-Ras augments transactivation by c-Jun. REF (0.5 μg per plate), NIH3T3 (0.5 μg per plate) or F9 (2 μg per plate) cells were transfected with the AP-1-dependent −73Col-CAT reporter in the presence or absence of pZIPneoRas (5 μg per plate) and RSV-c-Jun (0.5 μg per plate in REF and NIH3T3, 2 μg per plate in F9) or RSV-v-Jun (2 μg per plate in all cell types) expression vectors, as indicated. The cells were collected 24–48 h after transfection and the CAT activity determined. The values shown represent averages from three experiments in each of the cell types. As a control for specificity of the Ha-Ras effect, the glucocorticoid receptor (GR)-dependent reporter MMTV-CAT (1 μg per plate) was transfected into F9 cells in the presence of either 2 or 5 μg per plate of a GR expression vector[34] and 10^{-6} M dexamethasone in the presence or absence of pZIPneoRas (5 μg per plate). CAT activity was determined 24 h after transfection. The results are from one experiment done in duplicate.

METHODS. All the expression vectors and reporters used in these experiments have been described previously[5,6,34]. Primary REF cultures were prepared from day-14 Fisher rat embryos by established procedures[3] and were used between 1 and 3 passages. NIH3T3 and F9 cells were cultured as described[9,11]. Transfections were done as described[5,9].

a lesser extent. The effect of Ha-Ras was mediated through the AP-1 binding site: a −63Col-CAT reporter lacking the TRE (ref. 5) did not respond to either Ha-Ras alone or Ha-Ras with c-Jun (data not shown). Ha-Ras did not increase c-Jun expression because an RSV-CAT reporter controlled by the same promoter used to express c-Jun was not induced (data not shown) and, more directly, the rate of c-Jun synthesis was unchanged (Fig. 3*a*).

pZIPneo-Ras also augmented transactivation by c-Jun four- to sevenfold in NIH3T3 and F9 cells (Fig. 1). As reported[20,21], Ha-Ras alone led to a marked increase in AP-1 activity in NIH3T3 cells, but not in F9 cells. As F9 cells do not express c-Jun[9,11], it would seem that stimulation of AP-1 activity by Ha-Ras requires c-Jun expression. As shown earlier[29], v-Jun did not stimulate AP-1 activity in NIH3T3 cells, even in the presence of Ha-Ras, but in F9 cells v-Jun activated −73Col-CAT and was responsive to Ha-Ras. The basis for the different activities of c-Jun and v-Jun in these cells is not yet clear. Although v-Jun was reported to be a more potent activator than c-Jun, these results were specific for HeLa cells[30] and in three other mammalian cell lines (F9, NIH3T3, HepG2) and, most importantly, primary REF cultures, c-Jun is more potent than v-Jun[29-31].

We also tested the effect of Ha-Ras on another activator, the glucocorticoid receptor. In F9 cells, Ha-Ras had a marginal effect on glucocorticoid receptor activity (Fig. 1), but in NIH3T3 and REF cells Ha-ras inhibited activity (data not shown). This well documented inhibition[32] is likely to be mediated by induction of c-Jun and c-Fos[33-35]. Ha-Ras also had a marginal effect on GAL4/VP16 (data not shown) or GHF-1 (see Fig. 5) activity. Thus, as previously shown[18,20], Ha-Ras is not a general stimulator of transcription.

Stimulation of c-Jun activity is Fos-independent

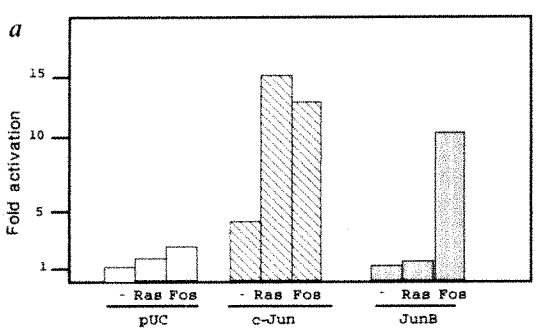
As transient expression of Ha-Ras induces c-*fos*[24], the stimulation of either endogenous AP-1 or transfected c-Jun by Ha-Ras could be explained by heterodimerization between c-Fos and c-Jun[36]. To assess the contribution of c-Fos to stimulation of c-Jun activity we first examined whether Ha-Ras affects the activity of JunB or JunD, both of which are highly dependent on c-Fos for transactivation[10,11]. Neither JunB (Fig. 2*a*) nor JunD (data not shown) were affected by Ha-Ras, although their activity was markedly enhanced by cotransfected c-Fos expression vector. We also examined two dimerization mutants of c-Jun. M15, in which two leucines in the c-Jun leucine zipper are replaced by phenylalanines, cannot form homodimers but can form heterodimers with c-Fos[37]. M3, in which Lys 288 thought to be involved in salt-bridge formation with c-Fos is replaced by a glutamate, is more active as a homodimer and is insensitive to further stimulation by c-Fos[37]. We find that although M15 is augmented by c-Fos, it is not responsive to Ha-Ras (Fig. 2*b*). M3, on the other hand, was insensitive to c-Fos but fully responsive to Ha-Ras. Therefore, the stimulation of c-Jun activity by Ha-Ras seen in these experiments is not mediated by c-Fos.

Ha-Ras stimulates phosphorylation of c-Jun

Ha-Ras is known to induce c-*jun*[23] and it was therefore possible that the augmentation of c-Jun activity resulted from induction of endogenous c-Jun protein which then acted with exogenous c-Jun. But Ha-Ras had no effect on the level of c-Jun expression in transfected REF (Fig. 3*a*) or F9 cells (data not shown). We therefore considered that Ha-Ras may stimulate c-Jun activity post-translationally. Cotransfection with Ha-Ras led to a considerable and specific increase in the level of c-Jun phosphorylation (Fig. 3*a*). As the phosphorylation of proteins trapped in the immunoprecipitates was not affected, the Ha-Ras effect on c-Jun phosphorylation is not due to increased uptake of ^{32}P-orthophosphate by the cells.

AP-1 activity is also elevated in Ha-Ras-transformed fibroblasts[20]. In the same cell lines used by Wasylyk *et al.*[20], Ha-Ras transformation leads to a specific 4.5-fold increase in c-Jun synthesis and a 35-fold increase in c-Jun phosphorylation (Fig. 3*b*). The stimulation of c-Jun phosphorylation is much higher than the general increase in protein phosphorylation in Ha-Ras transformed cells, which amounts to 2-fold for two of the background proteins present in the c-Jun immunoprecipitates. After normalizing for this general increase and its elevated rate of synthesis, we find that c-Jun phosphorylation is 4-fold higher after transformation by Ha-Ras.

Phosphopeptide maps (Fig. 4) indicate that the effect of Ha-Ras expression on c-Jun phosphorylation is site-specific. We find that Ha-Ras increases phosphorylation of sites x and y, located in the N-terminal half of c-Jun[17], at least 4-fold. Another N-terminal site, z, is not significantly affected. As reported[17], these sites contain only phosphoserines (data not shown). The effect of Ha-Ras on the three C-terminal sites, which are phosphorylated *in vitro by* GSK3 (ref. 17), is more complex. These sites reside within a single tryptic peptide whose triphosphorylated form corresponds to spot a, its diphosphorylated forms account for doublet b, and its monophosphorylated forms migrate as spot c. Ha-Ras led to a 2-fold increase in the mono- and diphosphorylated forms and no change in the triphosphorylated form. Assuming that these sites are phosphorylated by a single protein kinase[17], these changes are consistent with a simultaneous increase in the activity of this protein kinase and the putative phosphatase that acts on these sites. Activity of this phosphatase seems to increase after protein kinase C (PKC) activation and results in site-specific dephosphorylation and

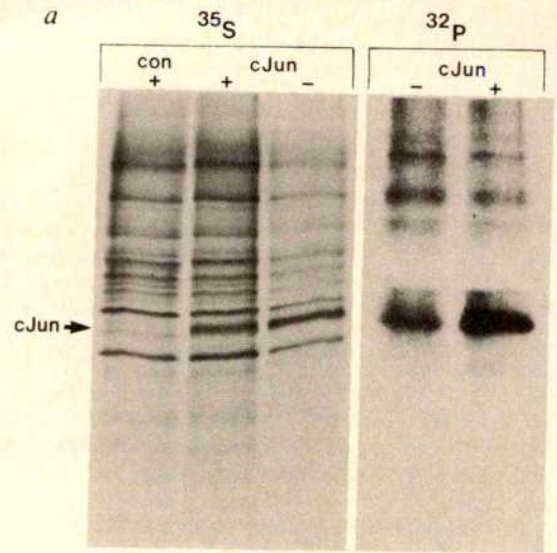


b

Leucine-zipper region

	280 287 294 301 308	Transactivation		
		-	+Fos	+Ras
c-Jun:	L EEKVKT L KAQNSE L ASTANM L REQVAQ L	3.2	9.4	10.5
M3	E	6.5	8.0	22.1
M15	F F	1.1	12.1	1.8

FIG. 2 Stimulation of c-Jun activity by Ha-Ras is independent of Fos. *a*, F9 cells were transfected with RSV-c-Jun (0.5 μg per plate) and RSV-JunB (5 μg per plate) expression vectors (these amounts of the c-Jun and JunB vectors were chosen because they led to similar levels of transactivation in the presence of c-Fos) in the presence or absence of Ha-Ras and c-Fos expression vectors (5 μg per plate) as indicated. Expression of cotransfected −73Col-CAT (2 μg per plate) was determined 24 h after transfection. Results represent the averages of three experiments. *b*, REF cells were transfected with wild-type c-Jun and the M3 and M15 leucine-zipper mutants (1.5 μg per plate) in the presence or absence of c-Fos and Ha-Ras expression vectors (3 μg per plate) as indicated. Expression of −73Col-CAT was determined 16 h after transfection and the results shown are the averages of three experiments. The sequences of the wild-type and mutant leucine-zippers are shown. Sequences of mutant leucine-zippers shown in single-letter amino-acid code.



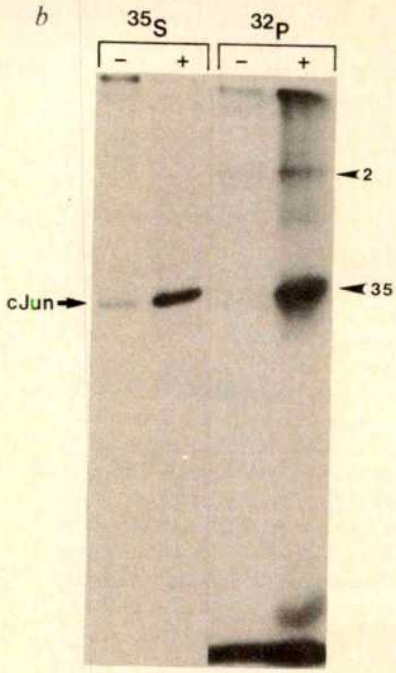


FIG. 3 Ha-Ras expression increases c-Jun phosphorylation. *a*, REF cells were transfected with either pUC18 (con) or RSV-c-Jun (7 μg per plate) in the presence (+) or absence (−) of pZIPneoRas (14 μg per plate). The cultures were labelled 12 h after transfection for 2 h with a mixture of [^{35}S]methionine and [^{35}S]cysteine (Tran[^{35}S]label). Parallel cultures were labelled with [^{32}P]orthophosphate for 5 h. The levels of c-Jun synthesis and phosphorylation were determined by radioimmunoprecipitation with a c-Jun-specific antipeptide antibody. *b*, Subconfluent cultures of FR3T3(−) and Ha-Ras-transformed FR3T3(+) cells were labelled for 2.5 h with either Tran[^{35}S]label or [^{32}P]orthophosphate. The levels of c-Jun synthesis and phosphorylation were determined by radioimmunoprecipitation of lysates derived from an equal number of cells, using a c-Jun-specific antipeptide antibody. The increase in phosphorylation of c-Jun and a protein that contaminates the c-Jun immunoprecipitates is indicated on the right-hand panel. These numbers come from three experiments in which the radioactivity in each band was directly quantitated using an Ambis β counter. Similar results were also obtained by densitometric analysis of the autoradiograms. METHODS. Transfected cell cultures on 100-mm plates were rinsed twice before labelling with serum-free labelling medium and incubated with either 5 mCi ml^{-1} [^{32}P]orthophosphate (ICN) or 150 μCi ml^{-1} Trans[^{35}S]label (from ICN) in 2 ml DMEM lacking either sodium phosphate or L-methionine, respectively. c-Jun proteins were immunoprecipitated from cells lysed in RIPA buffer (150 mM NaCl, 1.0% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, 10 mM Tris–HCl pH 8.0 and 0.1 mM PMSF). Lysates were cleared with preimmune serum and c-Jun protein immunoprecipitated with rabbit antiserum directed against a C-terminal peptide of human c-Jun corresponding to AA 316–331 (VNSGCQLMLTQQLQTF) as described[17].

elevated c-Jun DNA binding activity[17]. As the half-life of c-Jun (1.5–2 h; W. Boyle, personal communication) is considerably shorter than the labelling period (5 h), the effect of Ha-Ras on c-Jun phosphorylation is unlikely to be due to differential turnover. In addition, shortening of the labelling period to 2.5 h (Figs 5 and 6) did not affect the observed phosphorylation pattern. Other mapping experiments indicate that c-Jun is also hyperphosphorylated on sites x and y in Ha-Ras-transformed FR3T3 cells. Ha-Ras transformation also led to a shift of the GSK3 phosphorylation cluster towards its monophosphorylated forms (data not shown).

Effect of Ha-Ras on c-Jun activation domain

These results suggested that it is sites within the N-terminal half of c-Jun, the location of its activation domain[31], whose phosphorylation is stimulated by Ha-Ras. To determine whether

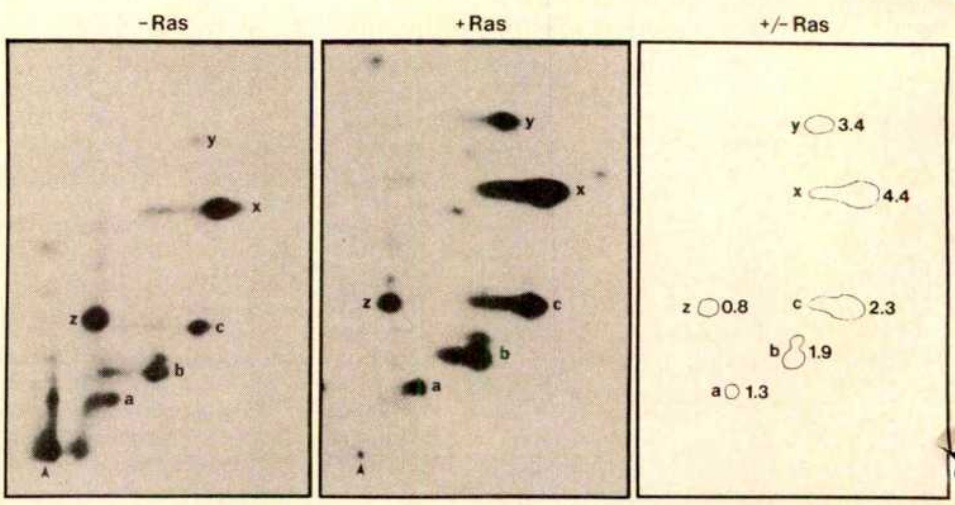
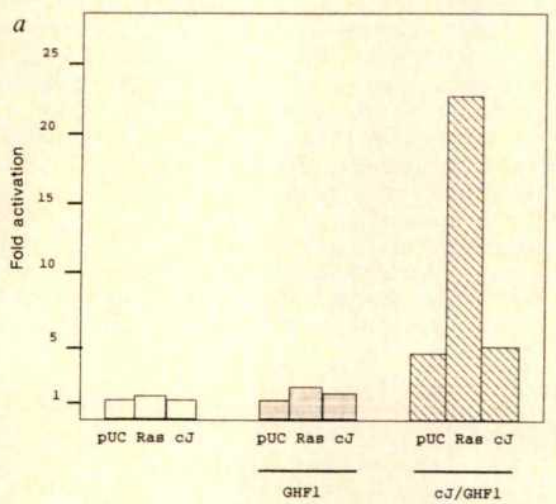
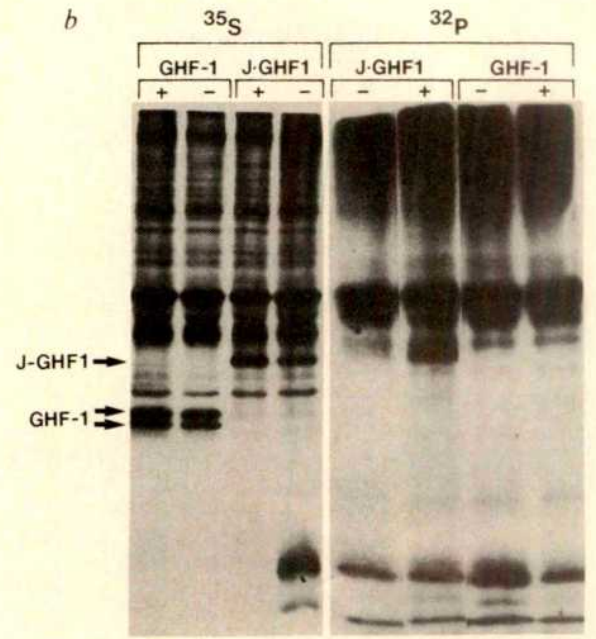


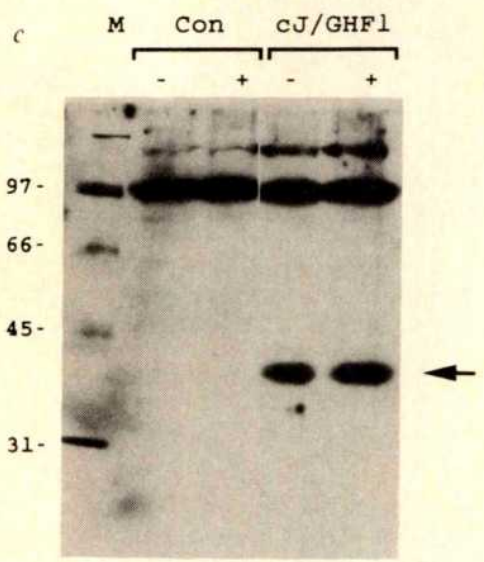
FIG. 4 Stimulation of c-Jun phosphorylation by Ha-Ras is site-specific. ^{32}P-labelled c-Jun protein was isolated from equal numbers of REF cells transiently transfected with RSV-c-Jun in the presence or absence of pZIPneoRas as indicated in Fig. 3*a*. After SDS–PAGE the c-Jun protein was eluted and subjected to tryptic digestion. The tryptic digests were applied to thin-layer plates and resolved in the horizontal dimension by electrophoresis at pH 1.9 (anode to the left) and the vertical dimension by ascending chromatography. The phosphopeptides were detected by autoradiography after which they were eluted and counted. The rightmost panel shows the ratio of ^{32}P content in each of the c-Jun phosphopeptides in the presence and absence of Ha-Ras expression. METHODS. Cell labelling and immunoprecipitation of ^{32}P-labelled c-Jun were as described above. Equal amounts of radioactivity were loaded on an SDS-polyacrylamide gel and after elution from the gel the labelled c-Jun protein samples were digested to completion with trypsin and subjected to two-dimensional phosphopeptide mapping as previously described[17].

Ha-Ras affects the c-Jun activation domain we used the chimaeric activator Jun-GHF1, in which the c-Jun activation domain is fused to the DNA binding domain of GHF-1, a pituitary-specific activator[30,38]. Ha-Ras expression increased activation of the −90GHF1-CAT(ΔAP-1) reporter by Jun-GHF1 5-fold (Fig. 5*a*). This stimulation cannot be explained by induction of endogenous c-Jun because cotransfection with RSV-c-Jun did not affect transactivation by Jun-GHF1. A reporter that lacks a GHF-1 binding site[39] was not activated by Jun-GHF1 even in the presence of Ha-Ras (data not shown). Under these conditions GHF-1 itself was a weak activator and only marginally responsive to Ha-Ras (Fig. 5*a*).

Stimulation of Jun-GHF1 activity by Ha-Ras correlates with increased phosphorylation of Jun-GHF1. Ha-Ras did not affect the rate of either Jun-GHF1 or GHF-1 synthesis (Fig. 5*b*), or the total amount of Jun-GHF1 (Fig. 5*c*), but it did cause a fivefold increase in Jun-GHF1 phosphorylation (Fig. 5*b*), although GHF-1 itself was not phosphorylated. The phosphopeptide maps in Fig. 5*d* confirmed that this phosphorylation occurred on sites derived from c-Jun. The sites phosphorylated







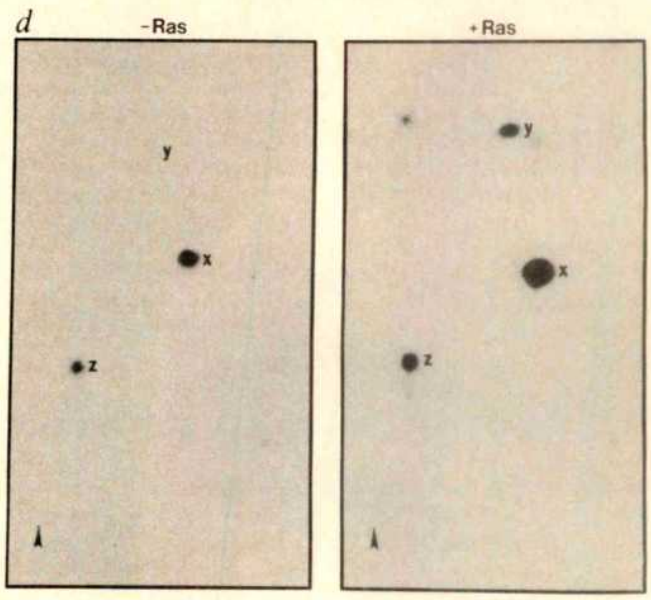


FIG. 5 Ha-Ras stimulates the activity and phosphorylation of the c-Jun activation domain. *a*, NIH3T3 cells were transfected with Jun-GHF1 and GHF-1 expression vectors (5 μg per plate each) and the −90GHF1-CAT(ΔAP−1) reporter (10 μg per plate) in the presence (+) or absence (−) of Ha-Ras or c-Jun expression vectors (5 μg per plate each) as indicated. The cells were collected 24 h after transfection and the levels of CAT activity determined. The values shown are averages of three experiments. *b*, F9 cells were transfected with Jun-GHF1 and GHF-1 expression vectors (7 μg per plate) in the presence or absence of pZIPneoRas (14 μg per plate). The cells were labelled 8 h after transfection for 2.5 h with either Trans[^{35}S]label or [^{32}P]orthophosphate. The synthesis and phosphorylation of Jun-GHF1 and GHF-1 proteins were examined by radioimmunoprecipitation of equal amounts of cell lysates using a GHF-1-specific antipeptide antibody[48], as described above. *c*, F9 cells were transfected with 10 μg of either pUC18 (con) or RSV-Jun-GHF1 (J.GHF1) in the presence (+) or absence (−) of 5 μg pZIPneoRas. Nuclear extracts were prepared 24 h later and analysed for Jun-GHF1 expression using anti-c-Jun antibodies raised against an N-terminal peptide. *d*, The Jun-GHF1 protein was immunoprecipitated from equal numbers of F9 cells transiently transfected with Jun-GHF1 expression vector in the presence or absence of pZIPneoRas and after SDS-PAGE was eluted and digested with trypsin. Tryptic digests were spotted on thin-layer plates and mapped as described above.

METHODS. The −90GHF1-CAT(ΔAP-1) reporter was derived from −90GHF1-CAT (ref. 38) by deletion of the *DraII-NdeI* fragment of pUC which contains a high-affinity AP-1 site. To analyse the level of Jun-GHF1 expression, nuclear extracts of transfected cells were prepared by established procedures[49] and 200 μg extract protein were separated by SDS-PAGE and electroblotted onto a nitrocellulose membrane for 3 h at 4 °C at 250 mA in 25 mM Tris, 192 mM glycine and 20% methanol. After blocking in 3% powdered milk, the membranes were incubated with anti-c-Jun antibodies directed against an N-terminal peptide and probed with [^{125}I]protein A (Amersham) at 0.4 μCi ml^{-1} as described[50].

in Jun–GHF1 are identical to the N-terminal sites of c-Jun, x, y and z, as verified by mixing experiments (data not shown). As before, Ha-Ras stimulated phosphorylation of sites x and y without significantly affecting site z (Fig. 5*d*).

To examine further whether the site of Ha-Ras action is the N-terminal activation domain of c-Jun, we used a c-Jun mutant deleted for its first 87 amino acids. This mutant, c-JunΔ1−87, retains only 10% of wild-type activity and was augmented only 1.5-fold by Ha-Ras compared to the fourfold stimulation of wild-type c-Jun (Fig. 6*a*). Although c-JunΔ1−87 was expressed at 50% the level of wild-type c-Jun, it was phosphorylated to a much lesser extent and its phosphorylation was no longer stimulated by Ha-Ras (Fig. 6*b*). Phosphopeptide maps of c-JunΔ1−87 indicated that it was no longer phosphorylated on sites x and y (Fig. 6*c*). In agreement with the lower level of GSK3 in F9 cells (J. Woodgett, personal communication), the GSK3 cluster was underphosphorylated in these cells and its phosphorylation state was no longer affected by Ha-Ras (compare Figs 6*c* and 4).

Discussion

Functional cooperation between two different oncogenes, as seen in REF transformation assays, is a well established phenomenon thought to be related to multistep carcinogenesis[40–42], but its biochemical basis is not well understood. Cooperation implies that either the two onocogenes function in complimentary pathways, or that one oncogene enhances the activity of the other[42]. In the case of c-*jun* and Ha-*ras*, which cooperate in REF transformation[3], the findings presented above are consistent with the action of Ha-Ras and c-Jun in a common pathway in which Ha-Ras affects upstream functions that control c-Jun activity, the downstream effector of this pathway. Stable and transient expression of activated Ha-Ras increases AP-1 activity[18,20,21], and although acute expression of Ha-Ras induces both c-*fos* and c-*jun*[23,24], transient induction of c-*fos* cannot account for the sustained increase in AP-1 activity seen in stably transformed cells[20]. In both transactivation or transformation assays, c-Jun is produced from an expression vector whose promoter is not affected by Ha-Ras, and so the effect of Ha-Ras on both functions must be mediated either by stimulation of c-Jun activity or by activation of a different pathway complementing c-Jun action. The experiments described here clearly demonstrate that Ha-Ras stimulates c-Jun activity and that this correlates with increased phosphorylation of c-Jun at two N-terminal sites, x and y.

Several observations suggest that sites x and y are within the c-Jun activation domain and that their increased phosphorylation is intimately associated with the potentiation of c-Jun activity. First, x and y are located within the part of c-Jun used

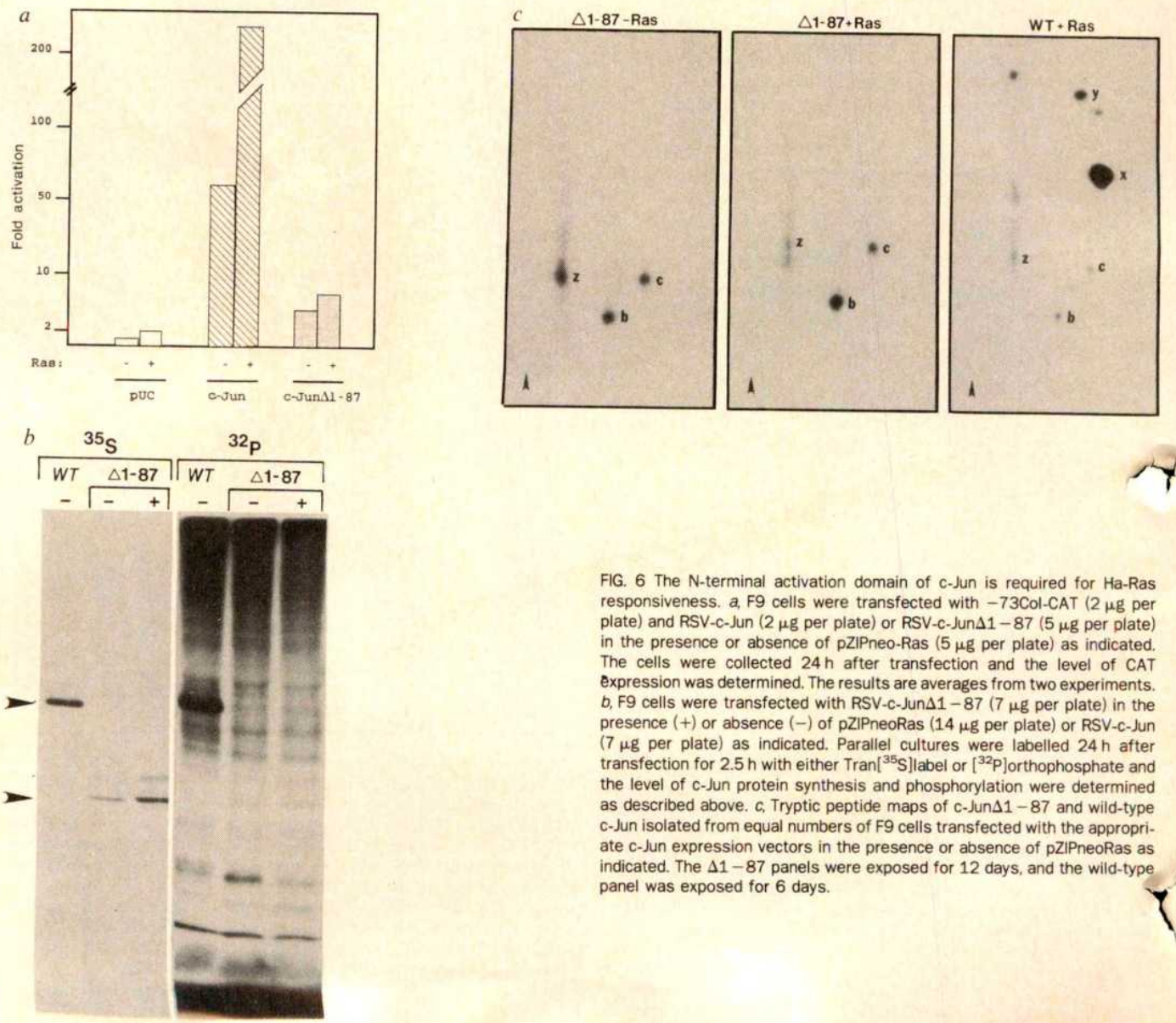


FIG. 6 The N-terminal activation domain of c-Jun is required for Ha-Ras responsiveness. *a*, F9 cells were transfected with −73Col-CAT (2 μg per plate) and RSV-c-Jun (2 μg per plate) or RSV-c-JunΔ1−87 (5 μg per plate) in the presence or absence of pZIPneo-Ras (5 μg per plate) as indicated. The cells were collected 24 h after transfection and the level of CAT expression was determined. The results are averages from two experiments. *b*, F9 cells were transfected with RSV-c-JunΔ1−87 (7 μg per plate) in the presence (+) or absence (−) of pZIPneoRas (14 μg per plate) or RSV-c-Jun (7 μg per plate) as indicated. Parallel cultures were labelled 24 h after transfection for 2.5 h with either Tran[^{35}S]label or [^{32}P]orthophosphate and the level of c-Jun protein synthesis and phosphorylation were determined as described above. *c*, Tryptic peptide maps of c-JunΔ1−87 and wild-type c-Jun isolated from equal numbers of F9 cells transfected with the appropriate c-Jun expression vectors in the presence or absence of pZIPneoRas as indicated. The Δ1−87 panels were exposed for 12 days, and the wild-type panel was exposed for 6 days.

to generate the Ha-Ras-responsive chimaeric activator Jun-GHF1. Second, deletion of the first 87 amino acids of c-Jun results in loss of these sites, decreases its phosphorylation and renders it insensitive to Ha-Ras. Third, the magnitude of the increase of c-Jun phosphorylation is very similar to the increase in its activity. The most parsimonious interpretation of the results is that phosphorylation of c-Jun at sites x and y augments its ability to activate transcription. In this respect c-Jun behaves in a similar way to its relative CREB, whose activity is stimulated by phosphorylation of a specific site within its activation domain by the cyclic AMP-dependent protein kinase[43]. Other less likely interpretations include an Ha-Ras-stimulated coactivator, or inactivation of a specific repressor that interacts with the c-Jun activation domain. Recent findings have suggested that c-Jun may be regulated by a repressor[30], but it is important to realize that evidence supporting its existence can be obtained only in HeLa cells and not in three other mammalian cell lines (F9, NIH3T3 and HepG2) and, most importantly, not in REF cultures. A Ha-Ras-stimulated coactivator is also an unlikely explanation because such a factor is expected to be a pleiotropic regulator and not c-Jun-specific. Yet, previous experiments indicate that Ha-Ras leads to specific activation of the TRE[18–22].

Expression of Ha-Ras has several effects on AP-1 activity. Initially it leads to transient induction of c-*fos* and c-*jun*[23,24] and increased phosphorylation of c-Jun. Elevated c-*jun* expression is maintained by positive autoregulation[16] but c-*fos* expression is transient[12,24]. Although the effect of Ha-Ras on c-Fos phosphorylation is unknown, a recent report indicates that increased phosphorylation converts c-Fos to an autorepressor contributing to its transient expression[44]. Therefore, most of the AP-1 activity in Ha-Ras-transformed cells is probably sustained by a phosphorylated form of c-Jun. This conclusion is supported by the transfection experiments which show that the Ha-Ras effect on c-Jun activity is not a result of c-Fos induction. Phosphorylation of other Jun proteins has not been analysed yet, but it is of interest that JunB is not stimulated by Ha-Ras (Fig. 2) and displays very weak transforming activity in the presence of Ha-Ras[45].

Regulation of c-Jun activity by phosphorylation is complex. Phosphorylation of c-Jun at sites next to its DNA-binding domain decreases DNA-binding activity and dephosphorylation of one of these sites by a putative PKC-activated phosphatase results in increased DNA-binding activity[17]. The present work suggests that phosphorylation of c-Jun at two N-terminal sites increases its transactivating potential. But full activation should depend on dephosphorylation of the inhibitory C-terminal site to allow DNA binding. TPA affects c-Jun DNA binding by inducing dephosphorylation of the C-terminal sites[17], but the effect of Ha-Ras is more complex. In addition to increasing phosphorylation of the N-terminal sites, Ha-Ras affects the state of the C-terminal phosphorylation sites resulting in accumulation of c-Jun forms that are underphosphorylated at this region and can therefore bind to DNA[17]. This effect of Ha-Ras is similar to that of TPA and is consistent with its ability to increase phosphatidylinositol turnover and PKC activity[46]. But, the effect of Ha-Ras on the N-terminal sites is unique and could be the result of its PKC-independent action[47]. Although further investigation is required to identify the Ha-Ras-activated c-Jun-kinase, the present work identifies a physiologically relevant substrate (c-Jun) that can be used to search for downstream targets of Ha-Ras action and to provide a biochemical explanation for oncogene cooperation. □

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Test of the decaying dark matter hypothesis using the Hopkins Ultraviolet Telescope

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SCIAMA has argued[1–4] that the dark matter associated with galaxies, clusters of galaxies and the intergalactic medium consists of τ neutrinos of rest mass 28–30 eV, whose decay generates ultraviolet photons of energy $\sim m_\nu/2 \approx 14$–15 eV. We have carried out a test of this hypothesis using the Hopkins Ultraviolet Telescope, which was flown aboard the space shuttle Columbia as part of the Astro-1 mission in December 1990. A straightforward application of Sciama's model predicts that we should have observed, from the rich galaxy cluster Abell 665, a spectral line from neutrino decay photons with a signal-to-noise ratio of ~30. We detected no such emission. For neutrinos (or any similar dark matter particle) in the mass range 27.2–32.1 eV, our observations set a lower lifetime limit significantly greater than Sciama's model requires.

The idea is based on work by several previous authors[5–7], but Sciama's theory significantly narrows the expected energy range of the decay photon and requires a lifetime for the decay of $\tau \approx 1-3\times10^{23}$ s, substantially shorter than lifetimes predicted in most standard theories of particle physics[8], but compatible with some left-right-symmetric models[9]. The severely constrained neutrino mass and lifetime result from Sciama's assumption that the decay photons are responsible for the partial ionization of the H I regions in the galaxy, as well as the ionization of the Lyman α clouds seen in the spectra of quasars at large redshifts. The short lifetime has the added benefit of making the hypothesis eminently testable.

The energy of the decay photons in Sciama's theory is only slightly above the ionization potential of hydrogen, or Lyman limit, at 13.6 eV. Consequently, a cluster of galaxies requires only a modest redshift to make the line emission expected from the decaying dark matter particles longer than the Lyman limit at 912 Å, where the interstellar hydrogen in our Galaxy becomes transparent. The Hopkins Ultraviolet Telescope (HUT) (Davidsen *et al.*, manuscript in preparation) has been optimized for spectroscopic observations in the 912–1,200-Å band (which is inaccessible to other space telescopes except for the low-resolution spectrometer on Voyager), and is therefore ideal for searching for the predicted neutrino decay line in clusters with redshifts $z \leq 0.5$. The full spectral range covered by HUT is 830–1,850 Å in first order. HUT also includes a large aperture (17 × 116 arcsec) which projects to a substantial volume within a typical cluster and therefore yields a strong signal for the predicted neutrino decay line.

We selected several rich clusters in the range $0.1 \leq z \leq 0.2$ for observation with HUT on the Astro-1 mission, but because of Spacelab system-level problems and a weather-related shortening of the mission, only one of these clusters, Abell 665, was successfully observed.

Abell 665 is the richest cluster in the Abell catalogue[10] and a luminous X-ray source[11,12]. A recent study[13] yields $z = 0.18144 \pm 0.00084$ and a velocity dispersion $\sigma = 1{,}201^{+183}_{-126}$ km s^{-1} for 33 galaxies that were found to be cluster members. We assume the projected mass density of the cluster, including both the luminous and dark matter, follows a King model[14] of the form $\mu(r) = 2r_c\rho_0(1+r^2/r_c^2)^{-1}$ with a core radius $r_c = 0.5$ Mpc (ref. 15). The central mass density is then $\rho_0 = 1.7\times10^8\,\sigma^2/r_c^2\,M_\odot$ Mpc^{-3} $= 1.0\times10^{15}\,M_\odot$ Mpc^{-3}. The total mass integrated over the large HUT slit, which covered 68 × 457 kpc at the centre of Abell 665, is $2.9\times10^{13}\,M_\odot$. (We assume $H_0 = 50$ km s^{-1} Mpc^{-1} and $q_0 = \frac{1}{2}$ throughout.)

The uncertainties in this mass estimate contribute a large fraction of the uncertainty in our final result. Although Oegerle *et al.*[13] find no evidence for substructure in their distribution of velocities for the galaxies in Abell 665, two separate dynamical systems with dispersions of 900 km s^{-1} and mean velocities separated by 400 km s^{-1} could reproduce their data. This would lower our mass estimate by a factor of two. On the other hand, the central density and the core radius are not tightly constrained by the available data, and the mass enclosed by the HUT slit could well be higher than we have assumed.

If the mass of Abell 665 is predominantly made up of decaying neutrinos, the luminosity of the cluster is $L = 4.0\times10^{54}\,M_{13}\tau_{23}^{-1}$ $(\varepsilon/14\text{ eV})^{-1}$ photons s^{-1} in a line at energy ε, where M_{13} is the total mass in units of $10^{13}\,M_\odot$, and τ_{23} is the decay lifetime in units of 10^{23} s. The expected width of the decay line is 11 Å (full width at half maximum, FWHM), or 22 pixels, assuming the neutrino decay emission fills the HUT aperture and that the neutrinos have the velocity dispersion quoted above for the cluster. With the mass obtained above, the predicted flux at Earth is $0.089\,\tau_{23}^{-1}\,(\varepsilon/14\text{ eV})^{-1}$ photons cm^{-2} s^{-1}. In the relevant energy range HUT has an effective area of 8.0 cm^2, leading to an expected count rate of 0.24–0.71 counts s^{-1} for Sciama's proposed range for the lifetime $\tau \approx 1$–3×10^{23}.

Abell 665 was observed with HUT for a total of 1,932 s on 9 December 1990. The aperture was centred at right ascension $\alpha_{1950} = 8$ h 26 min 24.6 s, declination $\delta_{1950} = +66°\,00'\,36.0''$, which corresponds to the position of the brightest cluster galaxy (W. Oergerle, personal comm.). Beers and Tonry give the median galaxy position[16] as $\alpha_{1950} = 8$ h 26 min 18.7 s, $\delta_{1950} = +65°\,59'\,58''$, and the centroid from the Imaging Proportional Counter on Einstein as $\alpha_{1950} = 8$ h 26 min 27.7 s, $\delta_{1950} = +66°\,01'\,07''$. The HUT slit was oriented at a position angle of 40° to include both the X-ray centre and the median galaxy position. Most of the

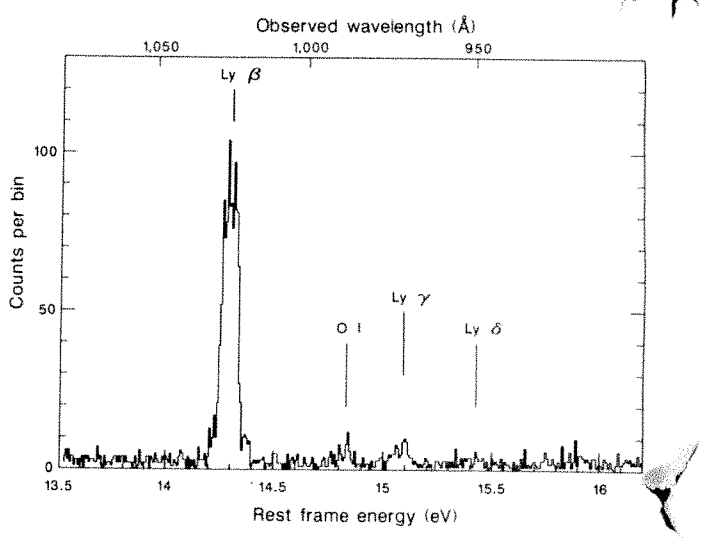

FIG. 1 Portion of the Abell 665 spectrum from the full 1,932-s observation covering the rest-frame energy range 13.6–16.1 eV. The ordinate is in observed counts per 0.51 Å bin. Prominent airglow features observed in other HUT spectra are marked.

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observation (1,378 s) was during orbital night, when airglow line emission is a minimum. The spectrum obtained had no features other than those expected from the airglow based on observations of other faint sources and blank fields during the Astro-1 mission. Figure 1 shows the relevant portion of the data from the full 1,932-s observation, corrected to the rest-frame of Abell 665. The positions of the known airglow lines Ly β, O I at 989 Å, Ly γ, and Ly δ are indicated. The observed flux of the Ly β line is 0.58 counts s^{-1}, essentially equal to the flux expected for the neutrino decay line. The background count rate (the apparent continuum in Fig. 1) of 1.2×10^{-3} per pixel per s is consistent with that from other blank sky observations. About one-third of this rate is due to internal detector background from charged particles (as measured with the aperture closed), and two-thirds is from grating-scattered geocoronal Ly α.

Emission lines from an extended source filling the $17''\times116''$ slit have a FWHM of 5.5 Å (11 pixels), as measured from other observations of bright airglow lines through this aperture. The Ly β line in Fig. 1 has a FWHM of 5.7 Å. As mentioned previously, emission from decaying neutrinos broadened by the cluster velocity dispersion would produce a line with FWHM 11 Å (22 pixels). At each pixel in the spectrum, we calculate upper limits to the intensity of an unresolved spectral feature at that location by computing the flux above the background in a surrounding 19-pixel region. This choice of size maximizes the signal-to-noise ratio for detecting a weak feature above the background and is conservative, as colder neutrinos would produce a narrower line which would be easier to detect. The 2σ upper limit is then $[\sum(C_i-B)+2\sqrt{\sum C_i}]/0.71t$, where B is the background count per pixel, C_i is the number of counts in pixel i, t is the total integration time, and the factor 0.71 is the fraction of the line flux contained in the 19-pixel region. Because this sum averages ~45 counts, the Poisson count distribution closely resembles a gaussian in its statistical properties, and we have simply used a factor of twice the Poisson standard deviation.

The only spectral features that exceed these upper limits during the orbital night portion of the observation are Ly β and Ly γ. In the full observation shown in Fig. 1, airglow features due to O I at 989 Å and Ly δ are also visible, and to be conservative we have used the higher fluxes seen for the whole observation in computing our upper limits. We have not yet developed a detailed model of the expected intensity of airglow features in the HUT spectra for the many relevant parameters, such as orbital position and viewing angle relative to the Earth limb and the Sun. Emission from decaying neutrinos could (fortuitously) lie at the same wavelength as Ly β or Ly γ, but as none of the airglow features are broader than the instrumental response, the dispersion of the decaying neutrinos in the cluster would have to be much less than the observed cluster velocity dispersion.

The Ly β line is bright enough that we can set a more stringent limit using the shape of the line profile and the Ly β/Ly α ratio from other observations. To be conservative, we compute the expected Ly β flux using the lowest observed ratio of Ly β/Ly α in the airglow observed by HUT. This comes from an observation of the Crab Nebula. (The Crab was within a few degrees of the anti-solar position on the sky, and the viewing angle during orbital night was almost entirely in the Earth's shadow.) We therefore presume that any contribution from decaying neutrinos to the observed Ly β in our observation of Abell 665 can be at most the amount by which the observed flux exceeds this minimum. Within three pixels of the centre of the Ly β line, we determine our upper limit by subtracting the observed Ly β/Ly α ratio of $(2.47\pm0.23)\times10^{-3}$ in the orbital night portion of the Crab Nebula spectrum from the ratio $(4.03\pm0.16)\times10^{-3}$ observed during orbital night on Abell 665. Scaling this to the Ly α intensity of 116 counts s^{-1} during orbital night in the Abell 665 observation, we obtain a maximum contribution to the Ly β intensity of 0.181 ± 0.033 counts s^{-1}. We use 0.247 counts s^{-1} as our 2σ upper limit within three pixels of the Ly β line centre. The Ly β/Ly α ratio observed in the Abell 665 spectrum is typical of that seen in other orbital night observations of the airglow. We may be able to reduce the upper limits at the Ly β line centre as we develop our understanding of the airglow spectrum.

To eliminate the possibility that the decay line could be hiding in the wings of geocoronal Ly β, we compare the Ly β line profile in the Abell 665 spectrum with that observed in other night observations of weak sources. Using a Ly β line profile obtained from observations of the Crab Nebula and the Perseus cluster, summed and smoothed with a three-point boxcar, we fit a 120-pixel region centred on Ly β in the Abell 665 spectrum, allowing the line centre, its intensity and a constant background to vary freely. The resulting $\chi^2=126.40$ indicates excellent agreement between the profiles of the template and the Ly β line of Abell 665. The shift in wavelength between the two lines is less than one pixel. We then add a second emission line at a fixed offset which is an integral number of pixels from the centre of the Ly β emission line. The intensity of the second emission line is increased until $\Delta\chi^2=4.2$ (95.4% confidence for one interesting parameter[17]) while all other parameters are re-optimized. This sets our 2σ upper limit in the regions 2–11 pixels above and below the centre of the Ly β emission line.

We have converted our upper limits on the decay-line intensity for Abell 665 to a lower limit on the lifetime of the neutrino, taking into account the variation in HUT sensitivity with energy (known to ±20% over the wavelength range of interest from laboratory calibrations and in-flight comparison of several white dwarf spectra to theoretical models; P. Bergeron, personal communication) and galactic extinction of $E_{B-V}=0.034$ for a galactic neutral hydrogen column density[18,19] of 4.7×10^{20} cm^{-2}. We use the extinction law produced by Longo *et al.*[20] from an earlier review[21]. Figure 2 shows the result, along with the range of lifetimes and energies predicted by Sciama's theory. Over most of the range of interest the observational limit on the lifetime exceeds 3×10^{24} s, a factor at least 10 times the value required by the decaying dark matter hypothesis. However, a narrow region of parameter space at $E=14.30\pm0.02$ eV, where interference from geocoronal Ly β is severe, would allow the theory to survive, if we have been so unfortunate as to choose a cluster whose redshift hides the neutrino decay line behind geocoronal Ly β. (The error in E is dominated by the uncertainty in the redshift of the cluster.) We expect that detailed analysis of the

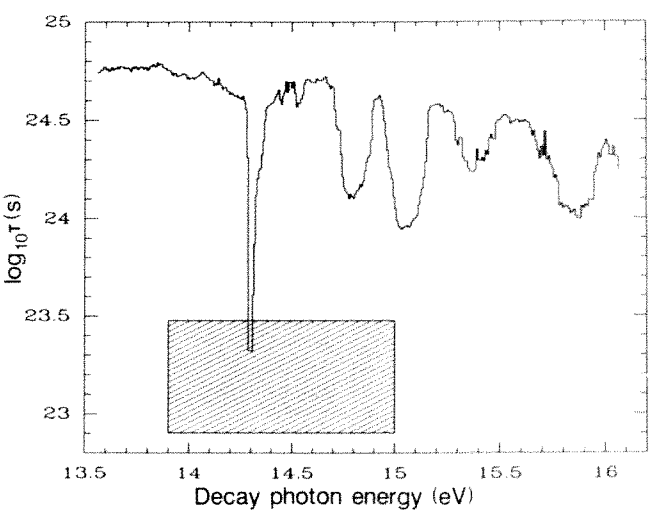

FIG. 2 Logarithm of the 2σ lower limits derived from HUT observations for the lifetime of decaying neutrinos (in s) as a function of the decay photon energy (in eV). The shaded region illustrates the range of energies and lifetimes compatible with Sciama's theory.

airglow data obtained by HUT throughout the Astro-I mission, together with further modelling, will allow us to strengthen our limit in the vicinity of Lyman β.

Highly sensitive observations of the cluster Abell 665 with the HUT thus fail to support the decaying dark matter hypothesis. The theory can survive only under one of the two following conditions: (1) the cluster is several times less massive than we estimate and the redshifted decay photon energy happens to coincide with the Ly β airglow line, or (2) there is substantial absorption of the decay line by previously unsuspected material along the line of sight. The latter has in fact been suggested by Sciama (personal communication) in response to our result. We believe we can rule out this possibility, but a discussion must be postponed to a future paper (G.A.K., A.F.D. and H.C.F., in preparation). Although neutrinos may yet provide the missing mass, they probably do not decay at rates high enough to explain the ionization balance in the interstellar or intergalactic medium. □

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Detection of binaries in the core of the globular cluster M15 using calcium emission lines

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M15 is the prototypical collapsed-core globular cluster. Having undergone collapse, its core is believed now to be expanding, with energy for the re-expansion provided by binary stars, which turn gravitational potential energy into kinetic energy[1]. Because these binary stars are generally more massive than single stars, they will have settled to the centre of the cluster[2]. We report here that several of the stars at the core of M15 show Ca II H- and K-line emission, characteristic of young, rapidly rotating stars and close binaries[3]. We argue that the emission from M15 comes from primordial binaries, in which a period of spin-up has led to magnetic field generation by enhanced dynamo action, which in turn causes heating of the stellar chromospheres. If this interpretation is correct, the Ca H and K emission may provide an important diagnostic tool of the binary population in cluster cores, and thus of the cluster dynamics.

Our observations were taken at the Isaac Newton Group of Telescopes on La Palma using the 4.2-m William Herschel telescope, equipped with the ISIS triple spectrograph, on the nights of 8, 11 and 13 August 1990. The blue camera and CCD-IPCS (charge-coupled device–image photon counting system) in combination with the H2400B grating gave us a dispersion of 8 Å mm^{-1} and a resolution of 0.4 Å (5 pixels full width half maximum, FWHM). Spatial sampling along the slit was 0.3″ per pixel, the final resolution at the detector was 1.1″ FWHM. Although we did not spatially resolve the three stars in the core, AC214, 215, and 216 (ref. 4), as shown in the contour plot in Fig. 1 from a blue Canada–France–Hawaii telescope CCD image taken in 0.4″ seeing (P.M.L. *et al.*, manuscript in preparation), the light at these wavelengths is dominated by the bluest objects. The seeing on the night of 8 August was 1.2″ to 1.5″. On the nights of 11 and 13 August the seeing was better than 0.7″ FWHM as measured on the spectrograph slit jaws.

Figure 2*a* shows a 40-min spectrum of a 3.0″ × 1.0″ area centred on the bluest of three stars in the central core: AC214 ($V = 14.75$, $B - V = 0.10$ (ref. 4)). Figure 2*b* shows the spectrum of the star AC623 ($V = 13.50$, $B - V = 1.04$ (ref. 4)), which is located 16″ from the centre. In both cases emission in the cores of the Ca II

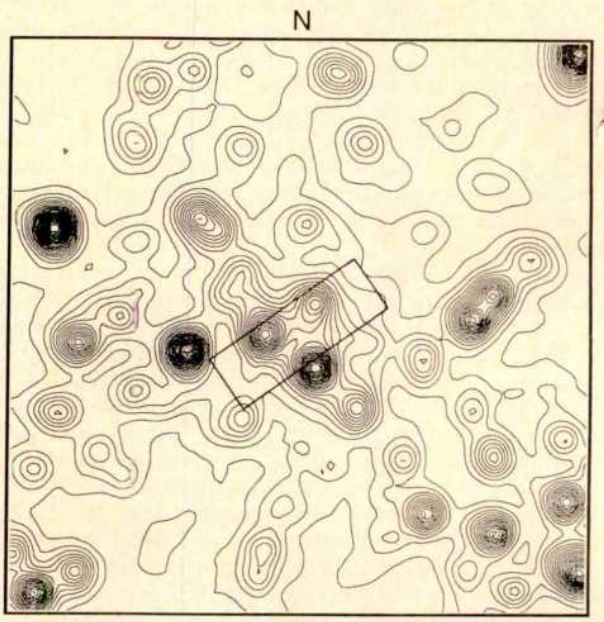

FIG. 1 A B-band image of the central 10″ × 10″ of M15 taken in September 1989 using the image-stabilization, high-resolution camera on the Canada–France–Hawaii telescope (P.M.L. *et al.*, manuscript in preparation). The pixel size is 0.11″. The box in the centre of the diagram shows the position of the slit for the spectra in Fig. 2*a*.

H and K lines is clearly detected. This line-core emission is produced by heating of the outer atmosphere to chromospheric temperature. The heating is controlled by the magnetic field created by the star's rapid rotation[3,5]. As a comparison the Sun, a moderately active star, only exhibits emission peaks up to a few per cent of its continuum level in surface-integrated light. Stars with emission peaks much larger than those shown in Fig. 2*a* and *b* do exist as well. FK Comae is an example of a giant exhibiting strong emission; it has a rotation period of 2.4 days[6]. It is believed that FK Comae is either a single star that coalesced from a close binary[7] or a close binary with a secondary star of low mass[8]. We have also included a spectrum of the star AC9 ($V = 14.16$, $B - V = 1.16$ (ref. 4)), located ~15″ from the centre of the cluster. This spectrum, shown in Fig. 2*c*, shows little or no emission. As the stars in M15 are ~15 Gyr old, magnetic braking should have reduced their zero-age main-sequence rotation rates sufficiently for little magnetic activity to occur. Two mechanisms that could reactivate old stars are (1) tidal capture in a close binary system and subsequent spin-up of the stars and (2) the existence and hardening of a large population of primordial binaries.

At the time of core collapse a globular cluster such as M15 is predicted to have a core density that may exceed $10^8\ M_\odot \mathrm{pc}^{-3}$ (ref. 9). At these high densities stars can no longer be assumed to act dynamically as point masses. It is likely that tidal interactions will occur, causing hard binaries to form (binaries with internal orbital velocities exceeding the local stellar velocity dispersion). When a main-sequence star is captured by a neutron star, its semi-major axis will be $\leq 3R_*$, where R_* is the radius of the main-sequence star. At these small distances tidal forces will cause the binary to synchronize stellar rotation with orbital motion, which results in the rotation rate of the star increasing from an almost negligible value to about once a day. This spin-up will promote the generation of magnetic fields by the dynamo action in the stellar interior, which will then result in heating of the outer atmosphere and emission in the Ca II H and K line cores. Evolving models using identical stars[10] give the number of tidally formed binaries near the time of core collapse as $\sim 10^3$. But calculations including both a degenerate component and a main-sequence star component show that the number of tidally formed binaries is ~30 near the time of core collapse[11]. Because we only observed post-main-sequence stars, the emission is unlikely to have been from tidally captured giants, as recent results[12] suggest that a giant's envelope would probably be completely disrupted in a close encounter with a degenerate star. Because it seems unlikely that sufficient numbers of giant-containing binaries could be produced by tidal capture we will not discuss this possibility further.

We now consider the possibility of primordial binaries. Recent evidence[13,14] indicates that as many as 10% of stars by number in globular clusters could be in the form of binaries. If this estimate is accurate then most of these are probably primordial binaries[13,14]. These binaries would have segregated to the core of the cluster fairly rapidly as they are heavier on average than typical single stars. After segregation, the central density of binaries will be five to ten times that of singles[2]. These binaries will harden because of three-body encounters with single stars, or they may strongly interact with one another, causing some to be ejected from the core and others to be completely destroyed. But overall, the remaining binary population will gradually harden[2], and the average orbital period (and hence the rotation period) will therefore decrease. Of course there will still be a large spread of the semi-major axes, but the average should lie between 0.2 and 0.5 AU[15]. This would reduce the periods of a large fraction of the binaries to small enough values to cause magnetic activity to commence, producing the observed Ca II H and K. The half-mass radius of the distribution of this binary population would be small[2], <0.5 pc. Thus, there will be nearly 15,000 binaries within 0.5 pc of the centre for a cluster of $6 \times 10^5\ M_\odot$. A conservative estimate is that 10% (1,500

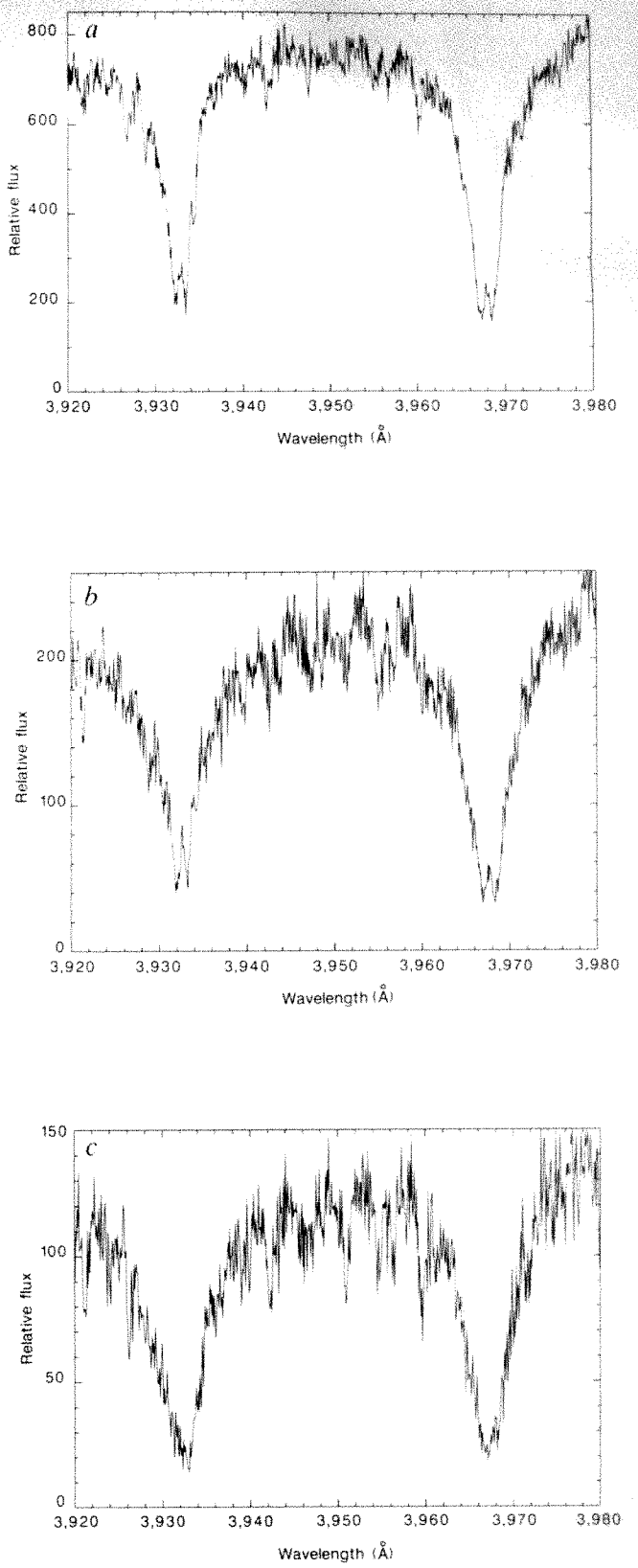

FIG. 2 Spectra taken with the William Herschel telescope. *a*, A 3.0″ × 1.0″ region (rectangle in Fig. 1) of the collapsed-core globular cluster M15. *b*, The star AC623 located 16″ from the centre of M15. Note the emission in the cores of the Ca II H and K lines. This emission rises to a peak of ~40% of the continuum. For comparison, the Sun exhibits emission peaks of Ca II H and K that are <5% of the continuum. This unexpected discovery from our August 1990 run may indicate the presence of binaries. *c*, The star AC9 located 15″ from the centre of M15. The line-core emission, if present, is much weaker than in the spectrum of Fig. 2*a* and *b*.

binaries) would show magnetic activity. Many of these binaries are likely to exchange their less massive components for heavier stars such as neutron stars, eventually producing low-mass X-ray binaries and millisecond pulsars[15].

Another mechanism to explain the Ca II H and K emission may be the dissipation of acoustic waves in the atmosphere of red giants or photospheric pulsations of stars on the giant branch[16–18]. This would also heat the atmosphere of the star, but is most likely to occur for stars near the tip of the red giant branch, and these stars would probably show significant mass loss. Two red giants in the globular cluster NGC6752 show Ca II K emission, and it has been suggested[19] that this is a pulsation instability producing shocks that lead to emission. One problem with this suggestion is that these stars would have radii of $\sim 100\ R_{\odot}$. If primordial binaries exist, then giants of this radius could not exist because they would be too close to their companion star. This may in fact explain why M15 and other collapsed-core clusters seem to be bluer in their cores than the surrounding area[20–22]. If stars near the main-sequence turn-off are prevented from becoming giants because they are in a close binary system, then red giants would be preferentially depleted in the core.

We conclude that the emission in the line cores of Ca II H and K lines that we found in many of the core stars of M15 may indicate the presence of synchronized close binary stars. If binary activity is the source of the emission, then it is likely that the stars are primordial binaries and not tidal-capture binaries. Because primordial binaries are expected to be more concentrated toward the core of the cluster, a radial dependence is expected in the ratio of stars showing Ca II H and K emission to those that do not. Previous searches for binaries in globular clusters have examined giants for radial velocity variations over extended periods[14]. Using Ca II H and K emission lines avoids this drawback because we can detect several close binaries in one observation. In future observations we plan to examine the radial dependence of this emission and conduct a survey of other globular clusters. If our interpretation of the emission as a result of binary activity is confirmed, spatially resolved, moderate-resolution spectroscopy could be a new diagnostic tool for determining the presence, distribution and relative numbers of binaries in globular clusters. □

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Cross-membrane coupling of chemical spatiotemporal patterns

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CHEMICAL systems may communicate by exchange of common species through mass transport, and such coupling may give rise to dynamical complexity beyond that possible in the independent systems[1–5]. We report here on dynamical behaviour arising from the diffusive coupling of chemical spatiotemporal patterns across a membrane. Chemical waves appear on Nafion membranes that are loaded with ferroin catalyst and bathed in a mixture of the reagents of the Belousov–Zhabotinsky oscillatory reaction. The waves on each side of the membrane couple by diffusive transport through the membrane. The coupling initially gives rise to the spontaneous appearance of spiral waves, and subsequent behaviour reveals several distinct phases of evolution, ultimately leading to complete spatiotemporal entrainment.

Figure 1*a* shows an image of regular wave behaviour on the ferroin-loaded Nafion membrane; the three horizontal lines correspond to grey-level profiles depicted in Fig. 2. Arcs defined by the intersection of these lines with a particular wave allow

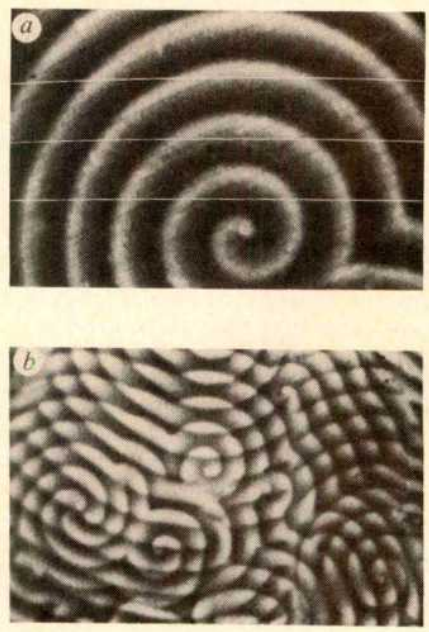

FIG. 1 Chemical waves propagating on the surface of a ferroin-loaded Nafion membrane suspended in a continuous-flow stirred-tank reactor (CSTR) (stirring rate 300 r.p.m.) pumped with Belousov–Zhabotinsky reaction mixture. These digital images[11,16] show the intensity of light (wavelength 500 nm) transmitted through a membrane 0.18 mm in thickness; field of view is 6.2×4.7 mm. The three horizontal lines correspond to the grey-level profiles in Fig. 2. Reactant concentrations in CSTR: $[NaBrO_3] = 0.19$ M, $[H_2SO_4] = 0.24$ M, [malonic acid] $= 1.9 \times 10^{-2}$ M, [ferroin] $= 5.8 \times 10^{-5}$ M, and [KBr] $= 8.7 \times 10^{-2}$ M; reactor residence time is 56.93 min; temperature maintained at 25.0 ± 0.2 °C. *a*, Nafion membrane loaded with ferroin to 16.7% capacity, giving strong coupling. The image was obtained 4.0 h after the reaction was initiated. *b*, Nafion loaded to 38.7% capacity, giving weak coupling. Image obtained 4.5 h after initiating the reaction. Note that the left-most spiral source is a spontaneous three-armed vortex.

media[13], which mimics the fast-slow dynamics of the Belousov-Zhabotinsky reaction[14]. Figure 4 shows one spiral on each side of the model membrane, evolving from uncoupled in *a* to fully entrained, although somewhat irregular, behaviour in *c*. An identical calculation with slightly higher coupling strength yields the more ordered behaviour in *d*, dominated by a single entrained spiral. Intermediate behaviour in *b* demonstrates the importance of relative wave orientation in the coupling, with ray-like domains of relatively uncoupled behaviour, and of partial and complete entrainment. The initially uncoupled spirals exhibit slightly different rotational periods (in a ratio of 11:12), and the higher-frequency spiral dominates throughout the evolution. The period of the entrained spiral in Fig. 4*d* lies between that of the initial spirals, reflecting the characteristics of both systems. These results indicate that the patterns observed in the experimental case result from spirals with slightly different frequencies on each side of the membrane. Other calculations for rotational periods in the ratio 1:2 show phase locking with

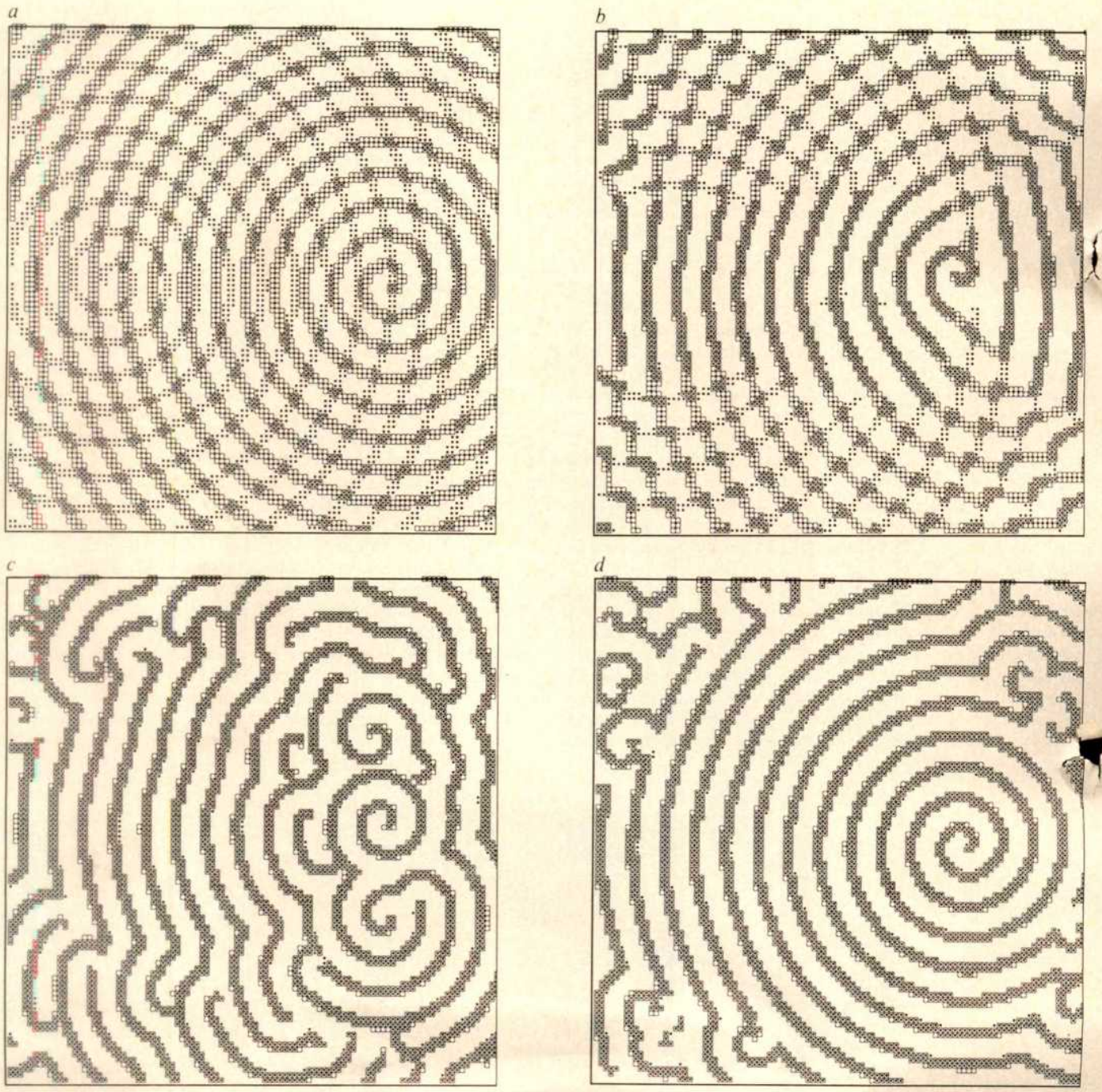

FIG. 4 Calculated evolution of spiral waves coupled across a model membrane. Open and filled circles show where oxidized catalyst is above 33% of its maximum concentration on each side of the membrane. The model for two coupled two-dimensional chemical-wave systems is based on a generic scheme for excitable media[13]: $\partial u/\partial t = f(u, v) + \nabla^2 u$, $\partial v/\partial t = g(u, v)$, where $f(u, v) = \varepsilon^{-1} u(1-u)(u - u_{th}(v))$, $g(u, v) = u - v$, and $u_{th} = (v + b)/a$. The two schemes are diffusively coupled by $\alpha(u_2 - u_1)$ and $\alpha(u_1 - u_2)$ terms for sides 1 and 2, respectively of the membrane, where α is transverse coupling constant. Diffusion in the plane of and across the membrane occurs only for the fast variable *u*, corresponding to bromous acid; there is no diffusion of the slow variable *v*, corresponding to the fixed catalyst. *a*, Initially uncoupled spirals. *b*, Interacting waves after 2,000 iterations. *c*, Entrained waves after 16,000 iterations. *d*, With stronger coupling: regular entrained spiral after 16,000 iterations. The transverse coupling constant $\alpha = 0.35$ in *a*–*c* and 0.45 in *d*. Parameter values: $a = 0.55$, $\varepsilon = 0.02$ and $b = 0.010$ and 0.016 for higher- and lower-frequency spirals. Grid: 200×200.

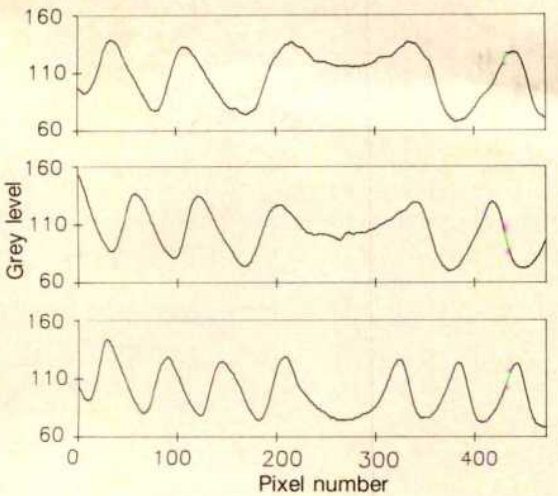

FIG. 2 Grey level as a function of distance for profiles corresponding to the three horizontal lines in Fig. 1*a*. Each pixel represents 12.1 μm.

calculation of the velocity normal to the front. Plots of wavefront position as a function of time are highly linear (a normal velocity of 0.163 ± 0.001 mm min^{-1} was calculated from Fig. 2 and subsequent profiles). These waves are much like those exhibited in thin films of Belousov–Zhabotinsky (BZ) solutions[6–8], but with shorter wavelengths and smaller propagation velocities.

The image in Fig. 1*b* shows a very different type of wave behaviour. Although successive images seem to suggest that the overall behaviour results from two sets of wave patterns evolving relatively independently, closer inspection shows that the two patterns are not independent. Their interaction is sufficiently weak, however, for the features to remain much the same for many hours.

The behaviour in Fig. 1*b* can be understood by considering the characteristics of Nafion, a perfluorosulphonic acid ionomer[9,10]. Partial ionization of covalently bound sulphonic acid groups gives rise to an overall net negative charge on the membrane, and negative ions do not significantly penetrate the resin matrix. The chemical-wave activity is therefore confined to the membrane surface because the anion BrO_3^- is an essential reactant. Studies of BZ chemical waves on polystyrene cation-exchange beads have clearly demonstrated that wave activity occurs on the surface of the resin matrix[11]. These considerations indicate that the waves in Fig. 1*a* also occur on both sides of the membrane; here, however, it is apparent that the wave activity is synchronized. The different behaviour in Fig. 1*a* and *b* results from strong coupling across the membrane in *a* and weak coupling in *b*.

The best candidate for the messenger species that mediates the coupling is bromous acid, the essential autocatalyst of the BZ reaction[8]. Bromous acid is a neutral molecule in the acidic reaction mixture and can diffuse readily through the membrane. A critical factor in the effectiveness of the messenger is the extent of ferroin loading: the entrained waves in Fig. 1*a* occur on a membrane in which 16.7% of the cation-exchange sites are occupied by ferroin; the relatively uncoupled waves in Fig. 1*b* occur on a membrane loaded to 38.7%. Higher loading apparently results in the interception of $HBrO_2$ in an oxidation–reduction reaction with ferroin[12], although other oxybromine species, such as BrO_2, might also serve as messengers and be similarly intercepted. Alternatively, it is possible that the diffusive transport of the messenger species is simply impeded by the bulky ferroin complex ions in the channels of the membrane.

The images in Fig. 3 show a system in which the wave behaviour is coupled over the course of many hours. Near the beginning of the experiment (Fig. 3*a*), faint semicircular patterns appear on each side of the membrane. The effects of cross-membrane coupling are clearly evident: spiral waves are spontaneously initiated at crossing points of the superimposed waves. The complex pattern of strongly coupled waves in Fig. 3*b* appears 1.0 h later. The pattern evolves to that shown in Fig. 3*c* 4.5 h later, with entrained but irregular behaviour displacing the cross-hatched patterns. The image in Fig. 3*d* shows complete entrainment and regular behaviour 31 h after initiation of the reaction.

These results can be viewed as the spatiotemporal analogue of coupled chemical oscillators[4,5]. In the strongly coupled system (Fig. 1*a*), the waves on each side of the membrane are entrained throughout. With weaker coupling (Fig. 1*b*), the patterns develop differently on each side of the membrane and seem at first to be independent, but become gradually entrained over long timescales (Fig. 3). Insights into the mechanism of entrainment may be obtained using a four-variable model based on two diffusively coupled two-dimensional chemical-wave systems. We use a computationally efficient scheme for excitable

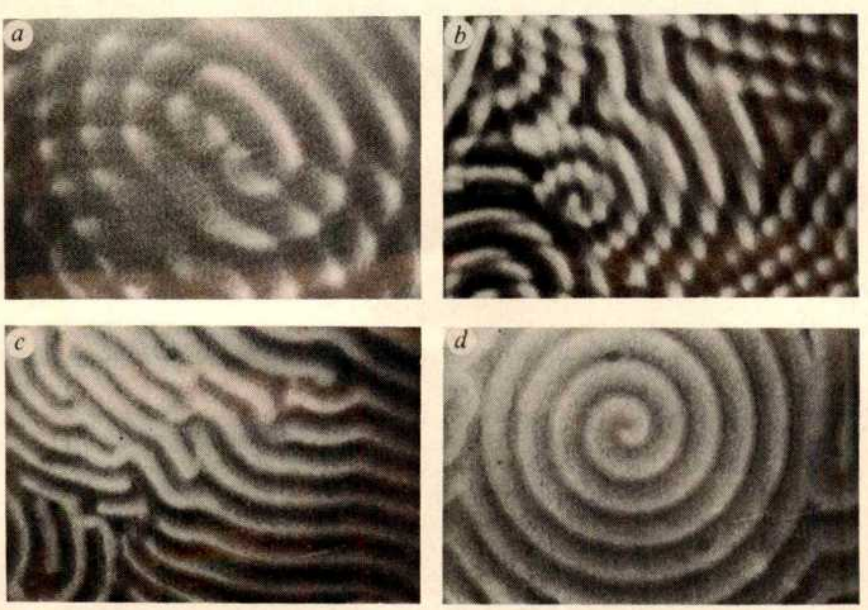

FIG. 3 Transition of spatiotemporal patterns from uncoupled to entrained; conditions are the same as in Fig. 1*b*. *a*, Behaviour after 5.5 h; *b*, 6.5 h; *c*, 11.0 h; *d*, 31.1 h. (The image of the spiral source in *d* was taken at a location near to that of the earlier images.)

1 : 2 oscillations interspersed with 1 : 1 behaviour and, at a slightly different coupling strength, phase death[4], where the wave patterns on one side of the membrane are completely extinguished by those on the other side.

Cross-membrane coupling of spatiotemporal patterns provides a new configuration for investigating communication between chemical systems. Transverse gradients of messenger species give rise to the spontaneous formation of spiral waves, which govern the subsequent evolution of entrainment (which includes the possibility of phase locking). Further studies of such cross-membrane coupling may provide insights into intercellular communication and coupling of excitable media in biological systems[15]. □

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Molecular nitrogen emissions from denitrification during biomass burning

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THE burning of biomass (forest vegetation, savannah grass, firewood and agricultural wastes) due to human activities in the tropics is an important source of nitrogen compounds in the atmosphere[1–4]. A recent experimental study[5] identified a gap of 35–60% in the nitrogen balance between its content in the fuel and that recovered in the ash and in gaseous emissions of NO_x, NH_3, HCN, CH_3CN and other nitriles, N_2O, higher-molecular-weight organic compounds and in the smoke. It was suggested that the missing compound had to be molecular nitrogen. We have now carried out appropriate experiments and find that molecular nitrogen is indeed the most important nitrogen species emitted from biomass burning, with the largest contribution coming from flaming combustion. The loss of nutrient nitrogen by biomass burning, which is ~10–50 Tg N yr^{-1} or 5–50% of global nitrogen fixation, may be particularly important for tropical ecosystems.

An evacuable, cylindrical stainless steel chamber (volume, 13.6 dm^3) with electropolished interior surfaces was used to burn different types of biomass in an artificial nitrogen-free atmosphere. Either helium–oxygen or argon–oxygen mixtures, 79 : 21% by volume, were used at ~1,200 hPa after evacuating and flushing the chamber several times. Side windows in the walls of the container allowed observations of the experiments for which 0.5–1 g of various kinds of biomass were placed in a metal sample holder and ignited by resistance heating with a current of 180 A (4 V) for 5–20 s. Sample holders were made of stainless steel wire mesh or tantalum sheet. The former construction provided a better oxygen supply than the latter. The amounts of N_2 and CH_4 produced and of O_2 consumed (only in $He–O_2$ atmosphere) were determined by gas chromatography using a thermal conductivity detector. CO_2 and CO were measured by non-dispersive infrared gas analysis. The amounts of nitrogen and carbon that were volatilized were calculated from the differences between their concentrations in both biomass and ash, which were determined by standard ultimate analysis.

To ensure that N_2 emissions are not due to the release of trapped nitrogen from the cell cavities of the fuel and to demonstrate the tightness of the system, we stored several samples of biomass in the evacuated chamber for 20 h and refilled with nitrogen-free test gas. Without ignition there was no detectable evolution of N_2.

Table 1 summarizes the results of our experiments. Biomass materials studied included wood carvings (0.08), savannah grass *Trachypogon* (0.59), pine needles (0.88), hay (1.3), freshly cut and dried green grass (3.8) and clover (4.6), with mass percentage N-fractions shown in parentheses. The carbon contents ranged from 43 to 51% with an average of 46 ± 3%. Moisture contents were in the range 3–6%. Biomass combustion in argon–oxygen atmospheres generally produced bright flames, in contrast to helium–oxygen mixtures, which produced more smouldering combustion, presumably because the thermal conduc-

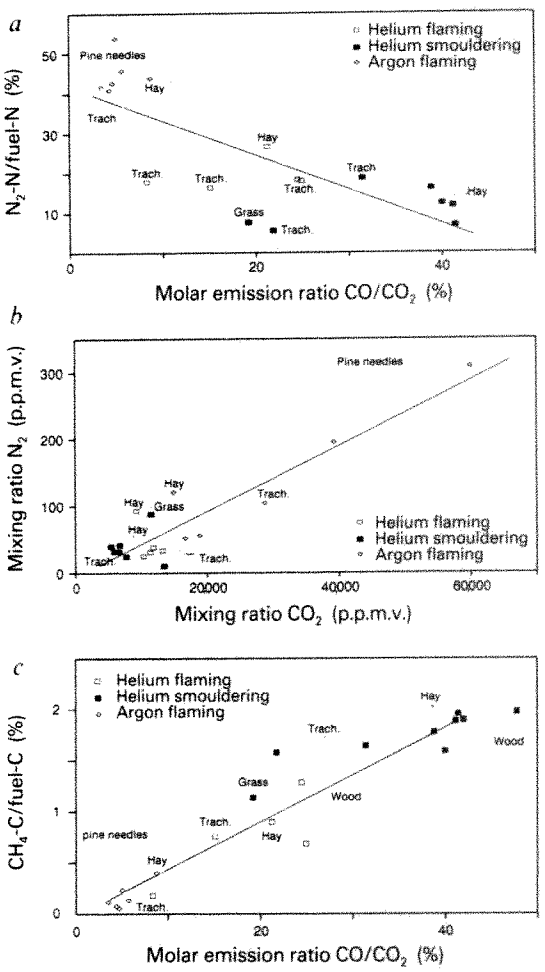

FIG. 1 Relations between N_2, CH_4, CO and CO_2 emissions observed in closed-chamber biomass burning experiments.

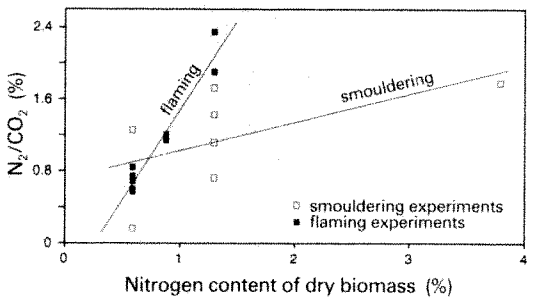

FIG. 2 N_2/CO_2 emission ratio in % (in terms of N/C) as a function of the biomass nitrogen content.

tivity and the associated heat losses are greater in helium. The thermal conductivity of argon is similar to that of nitrogen.

The combined emissions of CO, CO_2 and CH_4 do not fully account for the totally released carbon in most of the experiments. We ascribe this to the loss of material due to emissions of particulate matter and hydrocarbons not measured in this study which can, particularly in smouldering combustion, account for a large fraction of the products. The nitrogen balance is incomplete by necessity, as we do not report any nitrogen-containing compounds other than N_2.

From all experiments we find that, on average, $31 \pm 20\%$ of the volatilized nitrogen was converted to N_2. The N_2 yield of flaming combustion events was $46 \pm 15\%$ (total average). Compared with the fuel nitrogen, the N_2 yields were $23 \pm 16\%$ and $33 \pm 14\%$ (flaming combustions), respectively. Previously, we had shown that reduced compounds such as CH_4 are generated mainly during smouldering combustion, whereas the flaming stage results in higher oxidized products[3,5]. The CO/CO_2 emission ratio therefore serves as a powerful indicator for the extent of smouldering combustion. In our experiments the CO/CO_2 ratios varied over a wide range of 0.03–0.48. Figure 1 combines various relationships between fuel-N, N_2 and CH_4 emissions on the one hand, and CO and CO_2 on the other hand. In Fig. 1*a*, the relative amounts of N_2 (in % of the fuel N-content) are plotted against the CO/CO_2 ratio; in Fig. 1*b* the mixing ratios of N_2 are plotted against those of CO_2. Figure 1*c* shows the production of methane (in % of the fuel C-content) and its dependence on the CO/CO_2 emission ratio. The negative slope of the linear regression line obtained in Fig. 1*a* indicates that flaming rather than smouldering combustion is the main source of N_2. The plot in Fig. 1*b* shows a positive correlation and because CO_2 is formed primarily in the flame, this indicates again that N_2 is formed mostly during the flaming stage. Figure 1*c* shows a positive correlation between CH_4 and the CO/CO_2 ratio, which demonstrates that methane, unlike N_2, is formed mainly during smouldering combustion; this agrees with previous results[3,5].

Figure 2 shows the relation between biomass nitrogen content and the N_2 emissions. There is a clear dependence on the fuel N content with a correlation coefficient of 0.98 for data derived from experiments with a prolonged flaming phase. In contrast, the results from smouldering combustions show only a slight positive correlation with considerable scatter.

CO/CO_2 ratios measured during field[2] and laboratory[3] experiments are generally in the range of 5–15%. According to Fig. 1*a*, the corresponding yields of N_2 from fuel nitrogen are expected to vary between 40 and 30% with an average of ~36% corresponding to a CO/CO_2 ratio of 10%. This fraction, when added to that for other nitrogen-containing emissions (see the first paragraph), largely eliminates the gap in the nitrogen mass balance. Altogether, the measured and estimated emissions of the various forms of nitrogen, added to that remaining in the

TABLE 1 Carbon and nitrogen contents in biomass and ash and absolute emissions of CO, CO_2, CH_4 and N_2

			Elemental content				Emissions			
			Biomass		Residue		CO	CO_2	CH_4	N_2
No.	Biomass	Comment	(mg C)	(mg N)	(mg C)	(mg N)	(mg C)	(mg C)	(mg C)	(mg N)
1	savannah grass	He sm	243.2	3.18	38.2	0.93	14.8	47.1	4.01	0.59
2	savannah grass	He fl	247.2	3.23	50.1	1.00	23.0	92.1	1.69	0.58
3	savannah grass	He fl	302.7	3.95	71.7	1.43	23.5	96.0	3.90	0.72
4	savannah grass	He sm	263.9	3.45	68.8	1.61	23.9	109.7	4.18	0.19
5	savannah grass	He fl	217.7	2.84	65.7	1.36	12.4	82.1	1.66	0.47
6	savannah grass	He fl	276.3	3.61	127.0	2.24	8.9	107.3	0.49	0.64
7	savannah grass	Ar fl	310.4	4.05	28.9	0.69	12.5	219.8	0.41	1.85
8	savannah grass	Ar fl	182.7	2.39	16.1	0.41	6.8	146.3	0.09	1.02
9	savannah grass	Ar fl	187.0	2.44	18.4	0.40	6.0	138.8	0.13	1.00
10	savannah grass	He sm	201.9	2.64	1.4	0.04	NM	NM	NM	0.72
11	hay	He sm	219.0	6.58	31.6	0.80	25.2	60.9	4.29	0.45
12	hay	He sm	219.0	6.57	43.9	1.54	22.0	53.5	4.14	0.77
13	hay	He sm	164.3	4.93	15.5	0.62	21.5	53.8	2.62	0.61
14	hay	He sm	154.0	4.62	28.2	0.80	16.6	42.7	2.74	0.74
15	hay	He fl	213.7	6.41	44.4	1.19	15.5	72.9	1.92	1.70
16	hay	Ar fl	165.6	4.97	13.0	0.76	10.0	114.7	0.66	2.18
17	pine needles	Ar fl	756.6	13.05	210.8	5.20	15.7	454.6	0.82	5.45
18	pine needles	Ar fl	374.8	6.47	34.3	1.31	15.0	301.0	0.87	3.48
19	grass	He sm	252.1	22.28	28.6	2.94	18.0	93.8	2.86	1.68
20	grass	He sm	419.6	37.08	99.9	9.04	NM	NM	NM	2.15
21	clover	He sm	423.9	43.92	130.1	12.71	NM	NM	NM	1.39
22	wood	He sm	450.6	0.73	58.9	0.18	50.5	105.6	8.90	<0.18
23	wood	He sm	289.3	0.47	0.0	0.00	38.6	91.8	5.49	<0.18
24	wood	He fl	227.1	0.37	13.2	0.03	25.3	86.5	2.91	<0.18

He, helium atmosphere; Ar, argon atmosphere; sm, smouldering predominant; fl, flaming predominant; NM, not measured.

residue, now account for almost 90% of the fuel nitrogen. The rest may be explained by the uncertainty of the measurements and the variability of individual combustion processes. A closer inspection of higher nitrogen-containing compounds in gaseous and particulate states might be helpful in improving the balance even further. The present results make clear, however, that N_2 represents the single most important nitrogen emission from biomass burning.

We now consider the impact of N_2 emissions from biomass burning on the global nitrogen balance of the tropical biosphere. There are two ways to extrapolate worldwide emissions. The easiest way is to consider the total prompt release of CO_2 to the atmosphere by biomass burning in the tropics, which was recently estimated as 1.8–4.7 Pg C yr^{-1} (ref. 2). From our data in Fig. 1*b*, we obtain an average molar N_2/CO_2 emission ratio of 0.0047; the corresponding N/C mass ratio is 0.011. Combining these figures yields an estimated release of molecular nitrogen from global biomass burning of 20–52 Tg N yr^{-1} with an average of 36 Tg N yr^{-1}.

Alternatively, the release of N_2 can be estimated from the global burning of plant nitrogen, which is quite uncertain, but was estimated by Crutzen and Andreae[2] to be in the range of 15–46 Tg N yr^{-1}. Multiplying by the average yield of 30–40% of N_2 derived in the present study, yields a N_2 release in the range of 5–20 Tg N yr^{-1}. To check for consistency we can also compare the estimated N_2 emissions from biomass burning with emissions of $NO_y = NO + NO_2 + HNO_3$ from the same source. Our results suggest that ~36% of fuel nitrogen is emitted as N_2 compared with ~14% emitted as NO_y (ref. 5). Recent aircraft measurements[6] of several trace gases over West Africa during the bushfire season have provided vertical profiles of NO_y and CO in the troposphere, from which we deduce a molar ratio $NO_y/CO \approx 0.04$. The CO emission rate due to tropical biomass burning is 120–510 Tg C yr^{-1} (ref. 2). From the combination of these data we calculate a N_2 emission rate of 14–63 Tg N yr^{-1} with an average of 38 Tg N yr^{-1}, consistent with our first estimate presented above.

The current estimate for the global rate of terrestrial, bacterial nitrogen fixation is 110–170 Tg N yr^{-1} (ref. 7), and it is usually assumed that fixed nitrogen is lost entirely by microbial denitrification. The present results demonstrate that 12–47%, or alternatively 5–15%, of annually microbially fixed nitrogen is lost to the atmosphere in the form of N_2 due to 'pyrodenitrification'. The percentage is higher in tropical regions where ~90% of the global biomass burning takes place. Thus, the emissions of molecular nitrogen from biomass burning in these regions can cause important losses of fixed nitrogen to the ecosystems, especially from savannah and agricultural soils, reducing the availability of an essential nutrient for plants. □

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A possible normal-fault rupture for the 464 BC Sparta earthquake

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SURFACE ruptures have been identified for some normal-faulting earthquakes in the Aegean region[1–3], but for most historical earthquakes the associated faults are unknown. This hampers the evaluation of the rates and styles of present-day deformation, and the assessment of seismic hazard in the region[4]. Here we examine the famous earthquake that destroyed Sparta in 464 BC. Using SPOT satellite images and fieldwork, we have mapped a 20-km-long normal fault scarp trending approximately north–south, a few kilometres east of the ancient city. Our observations, combined with an examination of historical descriptions of the earthquake damage, suggest that the Sparta earthquake ruptured this fault scarp in an event of magnitude $M_s \approx 7.2$. The Holocene slip rate and the recurrence time for such large events on the Sparta fault would be ~1 mm yr^{-1} and ~3,000 yr, respectively.

To many historians of the Greek classical period[5–8], the earthquake that destroyed Sparta in ~464 BC was an important reference. Ducat[9] points out that there exist three independent contemporaneous descriptions of the 464 BC earthquake: by Thucydides, who reports the event in his History of the Peloponnesian War[5], and whose description has been followed by that of Pausanias[6]; by Ephorus, who apparently provided the source for Diodorus of Sicily[7]; and an unidentified hellenistic author, followed later by Cicero, Pliny and Plutarch[8]. The earthquake was large, according to Thucydides, who refers to it as the "great earthquake in Sparta" (ref. 5, I, 128). Diodorus gives an estimate of the death toll and an indication of multiple shocks: "the houses collapsed from their foundations and more than twenty thousand Lacedemonians perished. And since the tumbling down of the city and the falling in of the houses continued uninterruptedly over a long period, many persons were caught and crushed in the collapse of the walls" (ref. 7, XI, 63). Plutarch provides an idea of the local effects and details the devastation:

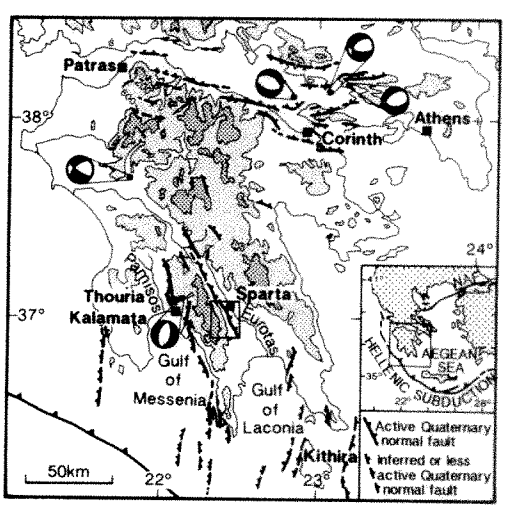

FIG. 1 Seismotectonic map of Peloponnesus (modified from ref. 1). Focal mechanisms from refs 1, 3. Insert shows the location of Fig. 2*a*; NAF denotes the North Anatolian fault.

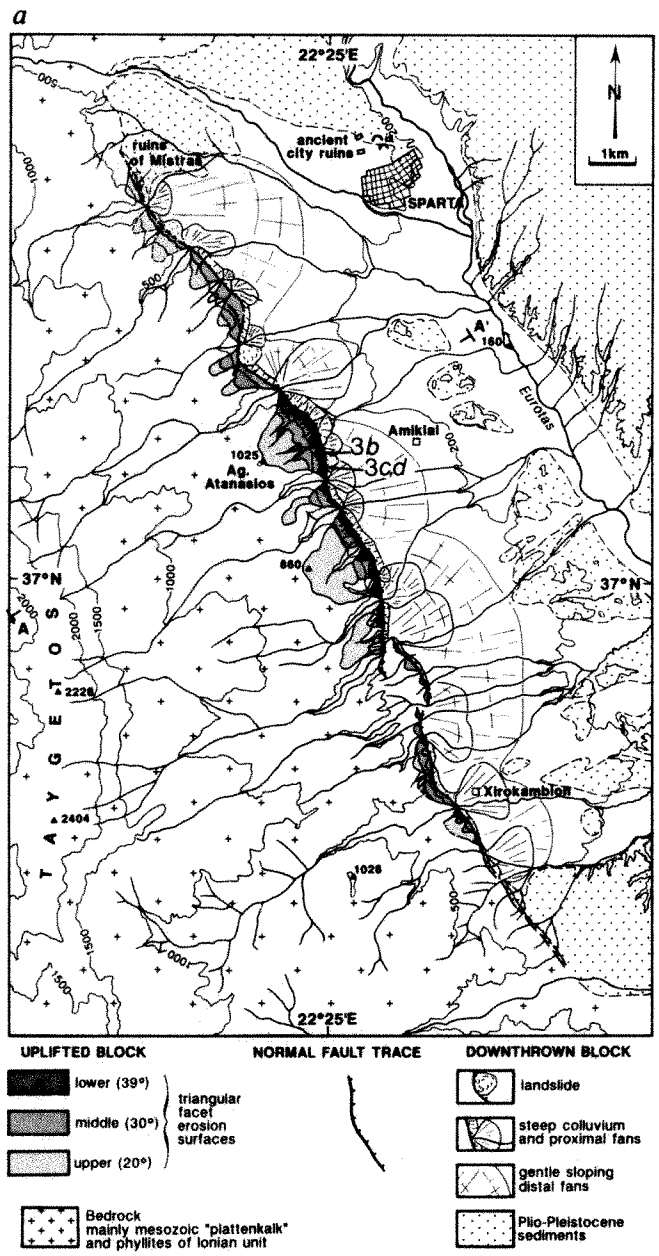

FIG. 2 *a*, Map of Sparta fault. Topography from ref. 21. Arrows are locations of Fig. 3*b* and *c*. *b*, Interpretative section at A–A'. Geology simplified from ref. 13. Total throw is probably greater than 2 km. Dashed fault is inactive.

"a greater earthquake than any before reported rent the land of the Lacedemonians into many chasms, shook Taygetus so that sundry peaks were torn away, and demolished the entire city with the exception of five houses" (ref. 8, XVI, 4). Plutarch also reports the existence, five centuries later, of "archaeological evidence" of the events: "Their tomb, even down to the present day, they call Seismatias" (ref. 8, XVI, 5). Clearly, the Sparta earthquake was large and destructive, probably accompanied by ground fissures, landslides, rock falls and other surface effects. This is compatible with a maximum intensity $I \geq$ X MSK (ref. 10) on the Mercalli scale. The death toll was probably very high, but the figure given by Diodorus (20,000 or about half of the inhabitants of the city[11]) seems to be overestimated[9,12].

The earthquake had immediate political consequences. Thucydides reports "the simultaneous revolt and secession to Ithome of the Helots and of some of the Perioeci (those of Thouria and of Aithaia)" (ref. 5, I, 101). According to Diodorus, "The Helots and Messenians ... stood in fear of the eminent position and power of Sparta; but when they observed that the larger part of them had perished of the earthquake, they held in contempt the survivors, who were few" (ref. 7, XI, 63.4). Although the importance of the revolt of the Helots of Laconia (southeast Peloponnesus) is not well established, all scholars agree on the significance of the revolt and secession of the Messenians (Helots and Perioikoi of Messenia, southwest Peloponnesus) after the earthquake: the so-called Third Messenian War[12]. The historical record thus strongly suggests that the destruction caused by the earthquake was much less in the Pamisos valley, where the Messenians were settled, than in Sparta and the Eurotas valley (Fig. 1). Given the short distance between the two valleys (Thouria is only 40 km west of Sparta), we infer that the 464 BC earthquake was not of subduction type but a shallow crustal event located not far from Sparta. A comparable example of local destruction is the normal-faulting earthquake that heavily damaged Kalamata in 1986[1].

The eastern front of the Taygetos range is bounded by an east-dipping, 60-km-long, normal-fault system made up of several left-stepping, NNW-SSE striking, en échelon segments (Fig. 1). This fault system is similar, but antithetic, to the west-dipping system formed by the Kalamata fault and the en échelon normal-fault segments located offshore of the Mani peninsula[1] (Fig. 1). The rock types that crop out on the Taygetos range near Sparta are mainly phyllites of Permian to early Triassic age and late Senonian–Eocene limestones of the Ionian unit[13]. These latter limestones mark remarkably well the normal-fault landforms (Fig. 2*a*), which are very similar to features s more clearly in other extensional environments such as the Bas and Range or Tibet[14–16]. East and south of Sparta, a 20-km-long fault segment with particularly young morphology[17] (here referred to as the Sparta fault) is composed of three sub-segments (Fig. 2*a*). The mountain front has a maximum local relief of more than 700 m at the central fault sub-segment (Fig. 2*b*). The front is formed by steep triangular/trapezoidal facets separated by distinct V-shaped valleys and 'wineglass canyons' (Fig. 3*a*). Three sets of triangular facets can be discerned, with maximum heights of about 725, 450 and 250 m, and slopes of ~20°, 30° and 40°, respectively (Figs 2, 3*a*). The decrease in facet heights towards the north and south defines the length and the individuality of the Sparta fault segment. The fault trace is linear, continuous and follows the mountain–piedmont junction (Fig. 2*a*). The large alluvial fans on the piedmont are mostly undissected and still active, but there is some young fanhead entrenchment, ~2–4 m deep near the fault trace. All of these observations suggest recent, sustained uplift of the front, and young faulting activity[18]. Furthermore, the existence along the three sub-segments of the Sparta fault of an almost continuous fresh scarp cutting across bedrock and indurated conglomerates (Fig. 3*b*, *c*, *d*) is definite evidence for recent, most probably seismic, faulting events. Although hanging-wall erosion may have increased the scarp height at some places, the uniform

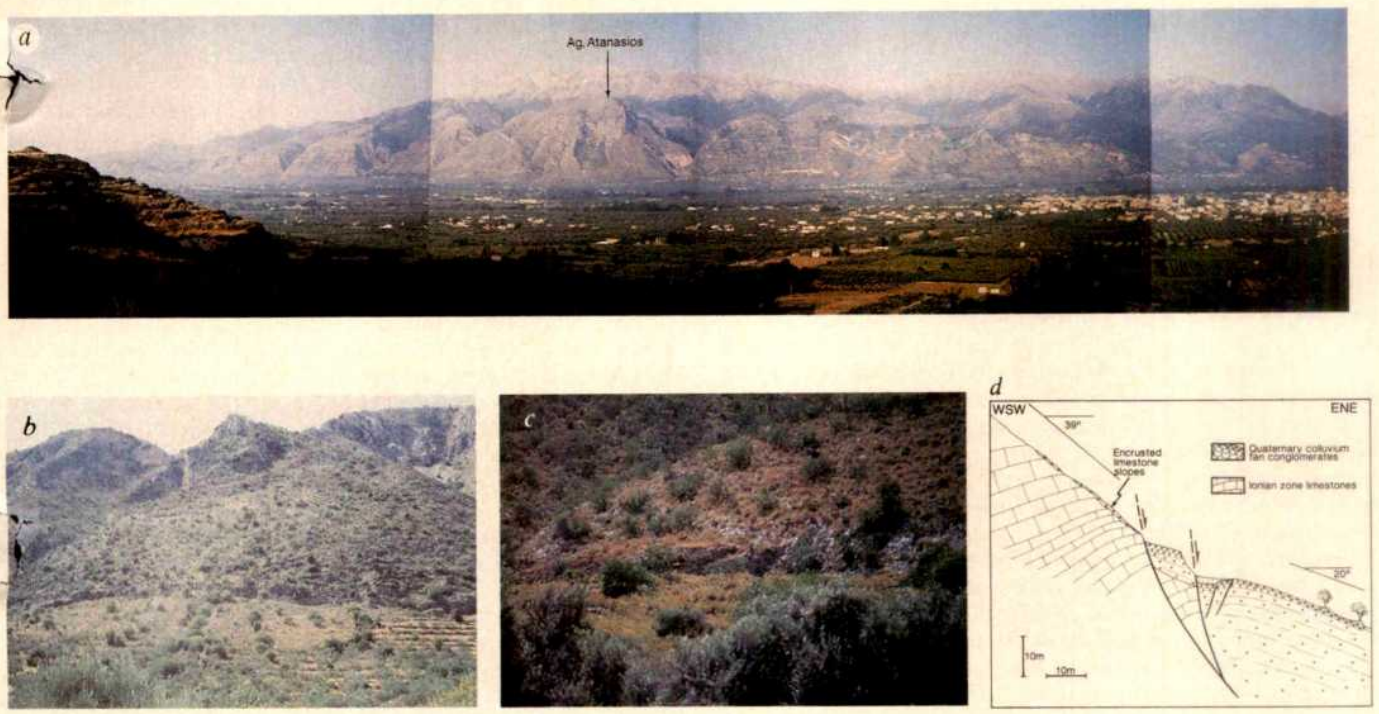

FIG. 3 *a*, Sparta mountain front. Panoramic view from the west. *b*, Fault scarp along base of triangular facet. *c*, Detail of scarp with uplifted conglomerates. *d*, Section of Sparta fault scarp at *c*.

shape of the scarp along strike implies a reliable maximum throw (vertical displacement) of ~10–12 m on the central fault sub-segment. Not having dug trenches, we cannot assess unambiguously whether this maximum throw corresponds to one or to many events. The observation, however, that a conglomerate wedge lies against the bedrock along a smaller inner scarp (~1/3 of the total throw), and that this wedge overhangs a main, fresher outer scarp (~2/3 of the total throw) (Fig. 3*c*, *d*), suggest that the total throw resulted from cumulation of at least two or three faulting events. In view of the historical record and of this field evidence, we suggest that the 464 BC earthquake could correspond to the last of these events.

To characterize a possible seismic rupture along the Sparta fault, we assume the fault geometry at depth to be the same as that of the Kalamata fault (Fig. 1), whose dip of ~45° is well constrained down to a depth of 10 km (ref. 1). Such a depth of the brittle/ductile transition zone seems to be implied by most studies of recent earthquakes in the region[19]. A rupture of the Sparta fault (20 × 14 km) with an average slip of 10 m (total displacement corresponding to the observed scarp) and shear modulus $\mu = 3.23 \times 10^{10}$ N m^{-2} would yield an earthquake with moment $M_0 = 9 \times 10^{19}$ N m. The large slip compared to the width of the fault would require a particularly high static stress drop (~17 MPa). An event with the same fault surface but a third of the displacement above yields a moment $M_0 = 3 \times 10^{19}$ N m, similar to the 1980 earthquake at Irpinia, Italy[20]. Our observations suggest that such an event is the most likely maximum-magnitude event along the Sparta fault. On the other hand, an average slip of only 0.5 m on the Sparta fault would yield a moment similar to those of the largest recent earthquakes of Thessaloniki (1978) and Corinth (1981) ($M_0 = 0.52$ and 0.68×10^{19} N m)[2,3]. We conclude that the 464 BC earthquake most probably had a moment M_0 of the order of 3.0×10^{19} N m and a magnitude $M_s = 7.2$. A comparison of the scarp along the Sparta fault with other scarps with similar morphology in southern Peleponnesus and Crete (R.A., H.L.-C. and D. Papanastassiou, manuscript in preparation) suggests that these scarps are of Holocene age (≥10 kyr). In this event the slip rate of the Sparta fault would be ~1 mm yr^{-1}, and, if at least three events produced the observed 10-m-high scarp ($M_s \approx 7.2$ each), the corresponding maximum recurrence time for large earthquakes would be of the order of 3,000 yr. □

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Aseismicity in the lower mantle by superplasticity of the descending slab

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It has been suggested[1–7] that deep earthquakes in subduction zones may be caused by mineralogical transformations in the subducting slab. These transformations, from olivine to modified spinel to spinel, are also thought to affect the rheology of the descending slab[2–4]. Experiments in the system Mg_2SiO_4–Fe_2SiO_4 (ref. 8) have shown that at still higher pressure, coinciding with the 670-km seismic discontinuity between the upper and lower mantle, spinel dissociates to a fine-grained mixture of perovskite plus magnesiowüstite. Here we argue that the dissociation product in the mantle will also be very fine-grained, and that its eutectoid texture, combined with the low temperature in the slab, will prevent grain growth. We note (as have others[9,3]) that fine-grained materials at moderately high temperatures and low strain rates exhibit superplastic behaviour; the resulting viscous behaviour of the slab below the spinel dissociation boundary may therefore account for the observed absence of seismicity below ~700 km depth, even if slabs do penetrate into the lower mantle.

Recent tomographic inversions of seismic data have indicated high-velocity anomalies that may correspond to a subducted slab penetrating into the lower mantle[10,11]. One objection to this idea of slab penetration is the widely observed lack of seismic activity below ~700 km depth. O'Connell[9] suggested that, among other possibilities, the aseismicity could be due to the low effective viscosity of the fine-grained mixture of oxides that he assumed would form by spinel dissociation in the lower mantle. Rubie[3] also pointed out that aseismicity of the slab in the upper mantle could arise from superplasticity associated with the fine-grained products of the transformations from olivine to modified spinel to spinel. Here we re-visit the idea of rheology-induced aseismicity, but with the benefit of new experimental constraints[8] on the nature of the post-spinel transformation.

The experiments of ref. 8 showed that mantle spinel (Fe/(Mg+Fe) = 0.1–0.15) dissociates at ~23 GPa and 1,600 °C into an assemblage of perovskite (Pv) and magnesiowüstite (Mw) within a very narrow pressure interval (less than 0.14 GPa). Sharp changes in seismic velocity and density observed at the 670-km discontinuity are reasonably attributed to this dissociation in the peridotitic mantle. The phase boundary has a negative Clapeyron slope of approximately −3 MPa °C⁻¹ (ref. 8). Therefore, the physical boundary of dissociation in the relatively cold slab should be convex towards greater depths (Fig. 1). This intrusion of low-density spinel into the lower mantle can produce a large buoyant force on the slab.

The product of spinel dissociation (Pv + Mw) is a fine-grained composite with a eutectoid texture (Fig. 2). In the course of dissociation, nucleation and growth of Mw (Pv) produces a zone of (Mg, Fe)SiO_3 ((Mg, Fe)O) around the nucleation site. Around this zone in turn, nucleation and growth of Pv (Mw) creates a zone of (Mg, Fe)O ((Mg, Fe)SiO_3). Thus the two phases should be distributed alternately in the product. We observe that the assemblage formed after 1 h at 1,600 °C and 24 GPa is an aggregate of grains 0.1–2.0 μm in size (Fig. 2). The corresponding product in the slab may contain smaller grains than this because of the lower temperature of the transformation (Fig. 1).

The deformation of such fine-grained materials at moderately high temperatures ($T/T_m > 0.5$, where T_m is the absolute solidus) and low strain rates results in superplastic behaviour[12,13], in which grain-boundary sliding is the dominant deformation mechanism. From experiments on fine-grained limestone, Schmit *et al.*[13] specified the regime of superplastic deformation. They showed that the flow stress at any given strain rate is sensitive to grain size in the superplastic regime, and that the expected flow stress for grain sizes less than 10 μm is extremely low; at $T = 0.54T_m$ and a strain rate of 10^{-10} s^{-1}, for example, the flow stress is 0.3 MPa. For a lower strain rate and a smaller grain size, the flow stress becomes lower still. Superplasticity in fine-grained ceramic oxides has been well established[14,15].

The assemblage of Pv and Mw produced in the slab satisfies the conditions for superplasticity: grain sizes of less than several micrometres, relatively high temperatures ($T/T_m > 0.5$; for Fig. 1, $T_m \approx 2{,}500$ K (ref. 16)) and low strain rates (~10^{-14} s^{-1} in the mantle). Grain coarsening of the assemblage would occur during further subduction. It should be emphasized, however, that at these strain rates, even grain sizes of ~100 μm may still produce superplastic behaviour[13,17]. Because Pv grains are interconnected (unlike the essentially isolated Mw grains; Fig. 2), grain-boundary migration of Pv will be the dominant mechanism of grain coarsening. We can make a rough estimate of the grain

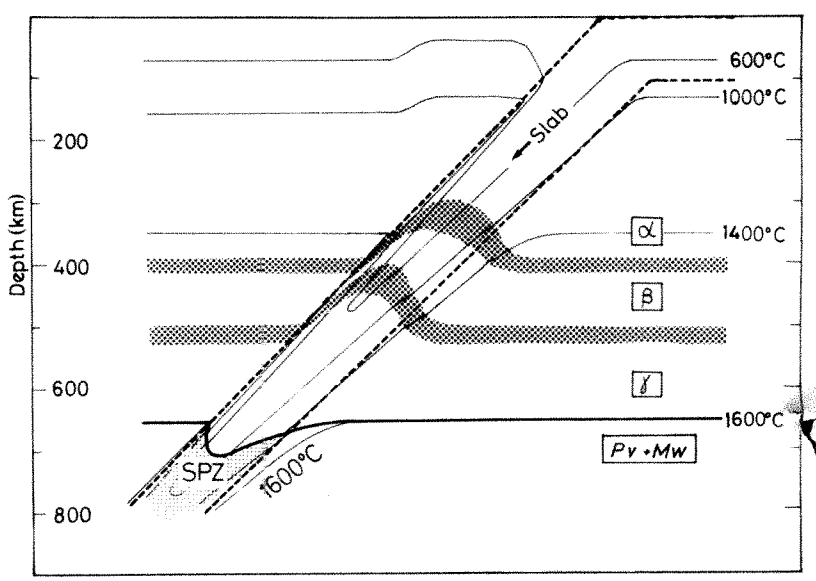

FIG. 1 Phase transformations in a descending slab. The upper mantle is composed of α (olivine), β (modified spinel) and γ (spinel) phases. The spinel dissociation into perovskite (Pv) and magnesiowüstite (Mw) takes place within a very narrow depth interval (bold line), whereas the α–β and β–γ transformations occur within the hatched areas. The mantle geotherm is from Sato *et al.*[26] and Ito and Katsura[27]. The temperature of the slab is calculated according to Schubert *et al.*[20]. Because of the positive Clapeyron slopes of the α–β and β–γ transformations, the transitional regions become shallower in the cold slab. (The possibility of a zone of metastable olivine in the slab[6,28] is neglected for simplicity.) The negative Clapeyron slope of the spinel dissociation at ~650 km depth causes the boundary to be at greater depths within the slab. Beneath this boundary, the fine-grained assemblage of Pv + Mw (region SPZ) satisfies the conditions for superplasticity. Therefore, earthquakes may occur only up to ~700 km depth in the slab. This is consistent with the maximum depth observed for earthquakes.

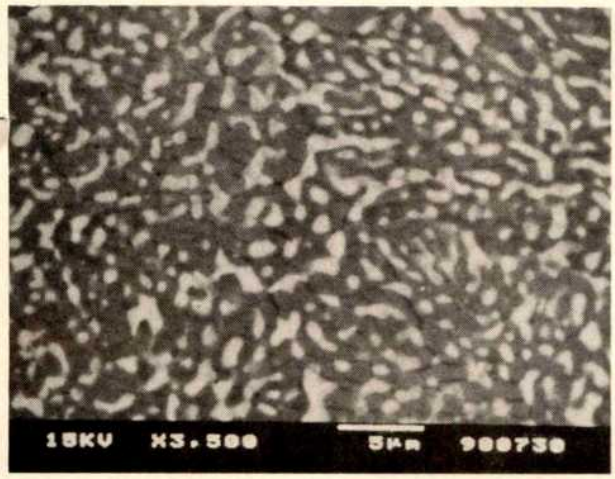

FIG. 2 Back-scattered electron image of the assemblage of perovskite (dark) and magnesiowüstite (light), showing a eutectoid (mutual intergrowth) texture. The sample was formed from a coarse-grained (10–20 μm) spinel (Fe/(Mg+Fe)=0.1) heated to 1,600 °C at 24 GPa for 1 h. The grain size of the composite is mostly less than 3 μm.

growth rate of Pv by the kinetic law[18] $d^3 \approx kt$, where d is the grain size at time t, and k, a growth-rate constant, is given by $k = k_0 \exp(-Q/RT)$ (k_0 is a pre-exponential factor, Q is an activation energy and R is the gas constant). Adopting $d \approx 1$ μm at $t = 1$ h (see Fig. 2), the time taken for grains to grow to ~100 μm is ~10^2 yr at 1,600 °C. As Q is several hundreds of kJ mol^{-1} for oxides (for example, $Q = 253$ kJ mol^{-1} for ZnO (ref. 18) and 580 kJ mol^{-1} for ZrO_2 (ref. 19)), the growth time in a slab could be several orders of magnitude larger than this because the temperatures are several hundreds of degrees lower than 1,600 °C (Fig. 1)[20]. The existence of a second phase (Mw) also suppresses grain growth substantially by a pinning effect[14,21]. Although superplastic flow in the slab can itself enhance grain growth[19,22], this effect should be negligible because of the small strain rate (10^{-14} s^{-1})[14]. Therefore an extensive superplastic zone should be formed in the slab beneath the spinel dissociation boundary, especially in the cooler region.

In this zone, the actual yielding stress is probably less than 1 MPa, so that virtually no elastic energy can be stored[13,17]. This may be the main reason why no earthquakes are observed in the lower mantle below ~700 km depth. The sudden disappearance of seismic activity at this depth is also consistent with the sharpness of the spinel dissociation boundary (width of less than 5 km)[8,23]. There is much debate about whether slabs penetrate into the lower mantle[24]; our work suggests that aseismicity below ~700 km is not necessarily evidence against slab penetration, as also suggested by O'Connell[9].

In the lower mantle there would exist a density contrast between the cold slab and the hot surrounding mantle. Ductility in the superplastic zone would, however, prevent the higher density of the slab from providing a driving force for subduction. In other words, the spinel dissociation determines the lower bound of the rigid body of the slab. The force acting on the slab then consists of a large negative buoyancy at depths above 400 km, owing to the density contrast from a large temperature difference (≥500 °C in some regions; Fig. 1) and to the transformation from olivine to modified spinel, and a fairly large positive buoyancy owing to penetration of the spinel zone into the lower mantle. The magnitude of these forces depends on the age, the dip angle and the descent speed of the slab. For the simple model shown in Fig. 1, the intermediate region of the slab between depths of 400 and 670 km should experience strong compression. This might account for the down-dip compressional earthquakes observed in seismic studies[5,25]. Negative buoyancy from a possible metastable region of olivine[6] will not change this conclusion. □

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Venom-conducting teeth in a Triassic reptile

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I REPORT here the discovery of highly distinctive reptilian teeth of early Late Triassic age from the Newark Supergroup of Virginia[1], which are distinguished by the development of a deeply infolded median groove on both the labial and lingual surfaces of the blade-like crowns. On the basis of the close structural similarity to comparable features in the two living species of the lizard *Heloderma* and on the poison-fangs in extant venomous snakes[2–5], it is suggested that these grooves functioned in venom conduction. This would represent the earliest instance of the use of oral toxins among reptiles (comprising diapsids and turtles) recorded to date.

The new fossils form part of a recently discovered assemblage of early Late Triassic vertebrates from the Richmond basin of the Newark Supergroup of Virginia (United States)[1]. The mostly disassociated but excellently preserved bones and teeth occur in a massive calcareous mudstone together with root traces, coalified plant debris, and numerous small calcareous nodules. The vertebrate assemblage is quite varied and includes several previously unknown taxa. The most common faunal element is the eucynodont synapsid *Boreogomphodon*, which is most closely related to certain genera from the Triassic of Brazil and Zambia.

Uatchitodon gen. nov.

Type-species. *Uatchitodon kroehleri* nov.

Etymology. *Uatchit*, the ancient cobra goddess of Lower Egypt, and ὀδούς (Greek; Ionic variant ὀδών), tooth.

Diagnosis. Tooth crowns strongly compressed labiolingually, recurved and typically with serrated anterior and posterior cutting edges. Both labial and lingual surfaces with deeply infolded median grooves.

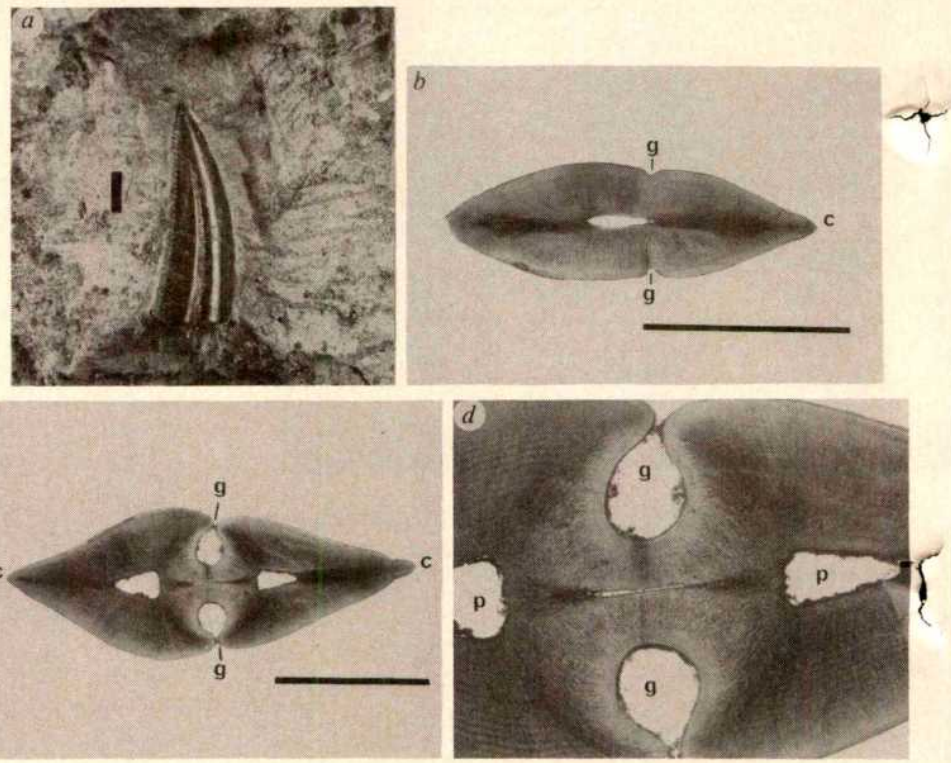

FIG. 1 *Uatchitodon kroehleri*, new species. *a*, USNM 448611 (holotype), isolated tooth crown as preserved in matrix. *b*, USNM 448612, transverse section near the tip of tooth crown; note shallow grooves (g), cutting edge (c) and undivided pulp cavity. *c*, USNM 448619, transverse section at mid-height of another tooth crown with deeply infolded grooves (g) on the labial and lingual surfaces and distinct cutting edges (c). *d*, Magnified detail of *c*; note modification of pulp cavity (p). Scale bars, 1 mm.

Uatchitodon kroehleri sp. nov.

Etymology. For P. A. Kroehler, who found the holotype and several other specimens.

Holotype. USNM (National Museum of Natural History) 448611, isolated tooth (Fig. 1*a*).

Referred material. USNM 448603–448610, 448621–448622 and 448624, teeth; USNM 448612–448619, thin-sections of tooth fragments.

Locality. USNM locality 39981 (1988–1), Chesterfield County, Virginia.

Horizon. Tomahawk Creek Member, Turkey Branch Formation, Newark Supergroup (Late Triassic: early middle Carnian)[1].

Diagnosis. Type and only known species of the genus as diagnosed above.

Only isolated teeth (mostly crowns) have been found to date but their singular structure sets them apart from those of any other known tetrapod. They reach an average crown height of about 10 mm but several fragments indicate the existence of larger teeth. The thecodont mode of tooth implantation indicated by the root of USNM 448624 suggests reference to the Amniota. Although the precise phylogenetic position of *Uatchitodon* remains to be determined, its teeth most closely resemble those of carnivorous archosauriform reptiles in overall form and structure and are unlike those of any Late Triassic synapsid. The pointed crowns are recurved and strongly flattened labiolingually (Fig. 1*a*). The posterior and the more apical portion of the anterior cutting edge are distinctly serrated on all but two teeth found to date, usually with six or seven denticles per millimetre. The thin enamel of the crowns is smooth except for some wavy banding on several teeth. Polished transverse sections of tooth crowns show numerous concentric growth lines in the dentine, which probably reflect periods of quiescence during the deposition of this tissue[2].

The diagnostic feature of the teeth is the presence of a deeply infolded median groove along both the labial and lingual surfaces of the crown. The groove tapers and becomes shallow toward the apex of the tooth and disappears before reaching the tip (Fig. 1*b*). Viewed in transverse (horizontal) section, it forms a deep invagination, which is lined by enamel (Fig. 1*c*–*d*). Together with the groove from the opposite side of the crown, it reduces the centre of the pulp cavity to a narrow slit for most of the tooth's height with the exception of the apical region (Fig. 1*d*). The anterior and posterior portions of the pulp cavity are distinct and more or less triangular in transverse section (Fig. 1*d*).

Each groove closely resembles the venom groove along the anterior edge of the poison fangs in many extant snakes, which forms a deep invagination[2]. In some taxa (for example, Elapidae), this groove subsequently becomes closed by apposition of its margins to form a tube[3]. The pulp cavity becomes distorted during the formation of this tube and assumes a lunate outline in transverse section. The maxillary and dentary teeth in the two living species of the lizard *Heloderma* have a deep lingual groove for venom delivery along the anterior margin of the crown and often a less prominent furrow along the posterior margin as well[4–5]. By analogy, the two grooves on the teeth of *Uatchitodon* presumably also served in venom conduction. The teeth of *Uatchitodon* differ from those of venomous squamate reptiles in the placement of the grooves on the labial and lingual surfaces of the crowns and in the presence of serrated cutting edges.

Features indicative of an oral venom delivery apparatus have previously only been reported in a single fossil amniote other than squamates. The therocephalian therapsid *Euchambersia* from the Upper Permian of South Africa has a deep lateral recess on the maxilla, which opens onto the palate behind the large canine. Grooves leading to the base of this tooth apparently transmitted products from a gland housed in the maxillary recess to a sulcus along the labial margin of the canine[6]. This represents the earliest example of an oral venom delivery apparatus in a tetrapod described to date.

The blade-like shape and the sharp, typically serrated cutting edges of the tooth crowns of *Uatchitodon* suggest a sectorial

function. The current lack of skeletal remains referable to *Uatchitodon* militates against specific palaeobiological inferences. Scenarios for the evolutionary origin of venom use in squamates have variously hypothesized oral toxins as a means to immobilize prey before ingestion, to aid in the digestion of the food bolus or to deter potential predators[5,7-9]. There exists evidence to support each of these functions but venom use probably originally evolved in a feeding context and was only subsequently co-opted for defensive purposes[5,9].

The inferred venom grooves in *Uatchitodon* predate the earliest record of such features among squamates[5] by over 100 million years. □

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Genetic evidence for the spread of agriculture in Europe by demic diffusion

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EUROPEAN agriculture originated in the Near East about 9,000 years ago[1-3]. The Neolithic reached almost all areas suitable for agriculture by 5,000 yr BP (before present)[2,4]. The routes and times of the spread of agriculture through Europe are relatively well established[2,3,5], but not its manner of spreading[6-8]. This could have been by cultural diffusion with few genetic consequences. By contrast, Ammerman and Cavalli-Sforza[2] proposed that the spread of farming increased local population densities, causing demic expansion into new territory and diffusive gene flow between the neolithic farmers and mesolithic groups. We have now tested observed genetic patterns against expectations derived from the demic expansion hypothesis. We found significant partial correlations of genetic distances with a distance matrix especially designed to represent the spread of agriculture on that continent, when geographic distances are held constant. These findings support the hypothesis of Ammerman and Cavalli-Sforza and invite further investigation into Renfrew's hypothesis on the origin of the Indo-European languages.

From 3,373 locations in Europe, we sampled 26 genetic systems (red cell antigens, plasma proteins, enzymes, histocompatibility alleles, immunoglobulins; Table 1). Details are specified elsewhere[9-11]. The number of localities ranged from 870 to 33 with a mean of 101. From these gene frequencies we computed Prevosti distances[12] separately for each genetic system, because different numbers of localities are available for each system.

A map of the onset of agriculture in Europe was obtained by pixel-by-pixel averages of spatially interpolated[13] dates for latest observed mesolithic and earliest observed neolithic settlements[2] (Fig. 1). The resulting 500-year contour classes were divided into 21 regions that approximate successive stages in the spread of agriculture into and through Europe. The regions were connected by a directed graph also shown (Fig. 1). Each node of this graph was assigned the date of the latest pixel in its region. But for clarity, the nodes are shown in the figure in the centres of their regions. For a given set of localities sampled for any one system, we constructed an origin-of-agriculture (OOA) temporal distance matrix between all pairs of localities. The OOA distance between any two localities depends on whether their regions constitute different bifurcating branches of the OOA graph. If so, the OOA distance is the sum of the temporal distances from each point to the node of its preceding region plus the shortest temporal distance between the two nodes along the graph. If not, the points are either in the same region or in regions constituting a unidirectional path in the graph. In these cases, their OOA distance is simply their temporal distance in the map of Fig. 1. We also computed geographic distance (GEO) matrices as great circle distances between pairs of localities.

As determined by Mantel tests[14,15], genetic distances are related to geography[16]. Matrix correlations range from −0.034 (for Lutheran) to 0.588 (for HLA-B). Overall significance (by Fisher's method for combining probabilities[17]) is $P \ll 1.0 \times 10^{-6}$. Necessarily (Fig. 1), the OOA distances are substantially correlated with the GEO distances (range from 0.502 for Rh haplotypes of system 4.13, to 0.711 for Se; all correlations significant at $P < 5.0 \times 10^{-6}$). The genetic distance and OOA matrices are also significantly correlated (for 16 genetic systems, significant correlations range from 0.072 for ABO, to 0.520 for HLA-A). The overall significance is $P < 5.0 \times 10^{-6}$.

The correlation between genetics and OOA could be due to their common dependence on geography. We therefore tested the partial correlation of genetics with origin of agriculture while

TABLE 1 Partial matrix correlation of genetic distances with origin-of-agriculture distances

System	$r_{(OOA,GEN).GEO}$	P
1.1 ABO	−0.16202	0.00000
1.2 A_1A_2BO	−0.02716	0.25240
2.5 MN	0.01793	0.68325
2.7 MN haplotypes	−0.06202	0.18177
3.1 P	−0.03435	0.23620
4.1 Rh	0.05448	0.99678
4.13 Rh haplotypes	−0.09464	0.04233
4.19 Rh haplotypes	−0.06856	0.11137
5.1 Lu	0.01491	0.55992
6.1 K	0.02275	0.70664
6.3 K (anti-K, −k)	−0.03673	0.32844
7.1 Se	−0.07997	0.13861
8.1 Fy	0.06725	0.92810
36.1 Hp	−0.04730	0.11253
37.1 Tf	0.23657	0.99834
38.1 Gc	−0.00321	0.39998
50.1.1 ACP1	0.21616	0.99962
52 PGD	0.01555	0.56605
53 PGM1	0.23784	0.99996
56 AK	−0.07062	0.13322
63 ADA	−0.02580	0.30443
65 T	−0.08506	0.05936
100 HLA-A	0.25741	0.99994
101-102 HLA-B	0.19968	0.99869
200 Gm 1,2,5	0.07732	0.81725
201 Km	−0.06247	0.27095

Geographic distances held constant. P is the left-tail cumulative probability based on the asymptotic approximation to normality. Thus all systems with $P \geq 0.95$ are significant at a one-tailed 5% level. Numbers preceding the system symbols, up to 65, are those assigned by Mourant *et al.*[28]; those from 100 and above were assigned in our laboratory. r, Partial correlation coefficient.

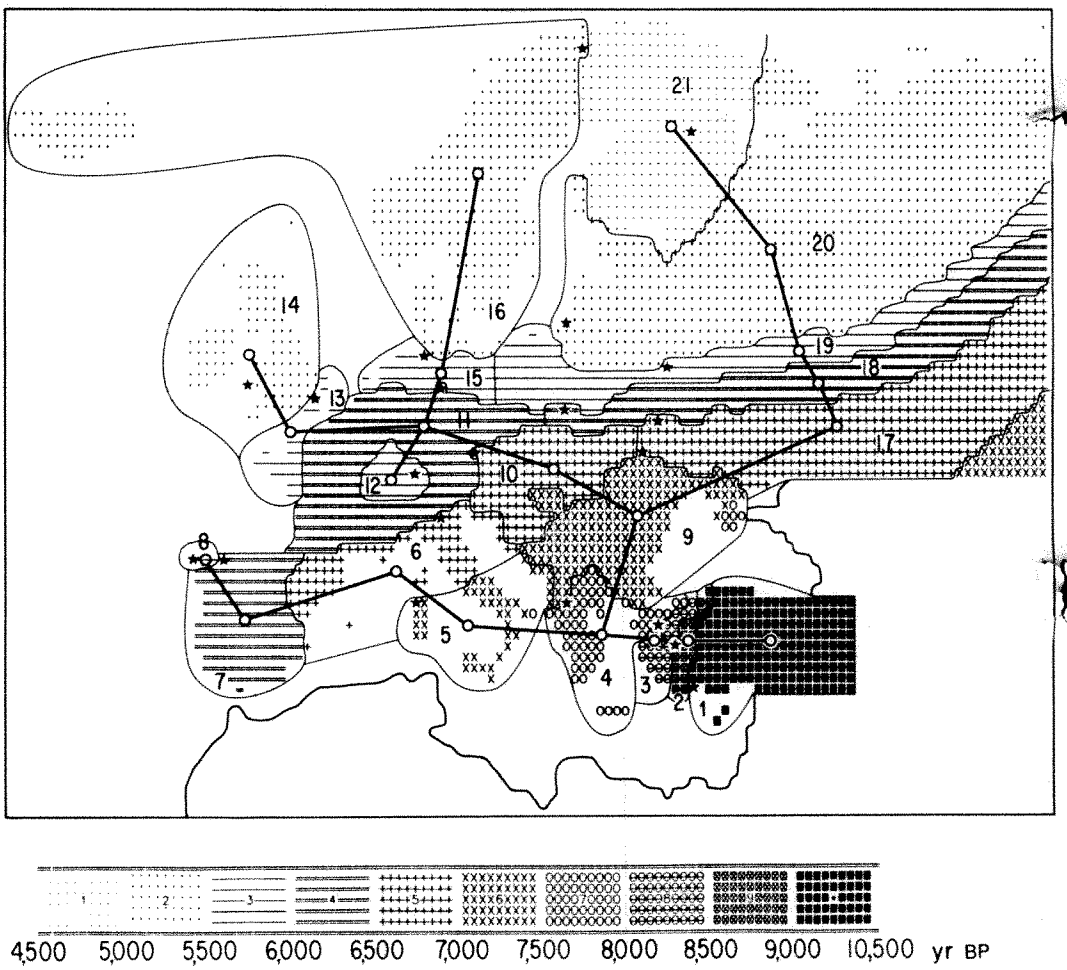

FIG. 1 Contour map of the onset of agriculture. The contours represent 500-year intervals identified in the key. The area has been subdivided into 21 numbered 500-year regions in which the latest pixel is marked by a star. For further explanation, see text.

keeping geographic distances constant (Table 1). When tested for significance by the Smouse-Long-Sokal test[18], six of the partial correlations (Rh system 4.1, Tf, ACP1, HLA-A, HLA-B, PGM1) remain significant at $P < 0.003$. Significance over all systems is very high ($P < 1.0 \times 10^{-6}$). There is clear evidence of a correlation between genetics and OOA above and beyond the predicted effects of geography[19]. Thus, our results support the demic diffusion hypothesis. Additional support comes from northwest-southeast clines predicted by Fisher's[20] model of a population wave of advance and demonstrated in a variety of ways[11,21,22].

Why should only 6 of 26 genetic systems show significant agreement with demic diffusion? Unless the allele frequencies of the immigrant farmers differed substantially from those of the native hunter-gatherers, there would be no trace of the migration and diffusion patterns. Sokal *et al.*[23] were unable to detect differences in gene frequency between immigrants and residents of less than 0.4 in simulated surfaces for 10,000 individuals. Although we cannot directly extend these findings to the human populations in our study, we note that only differences between major races show gene-frequency differentials of such a magnitude, and even then for only a few loci. When compared for over 60 alleles in Roychoudhoury and Nei[24], Japanese differ in only two allele frequencies by 0.4 or more from those of Italians; Yorubas from Nigeria differed that much from Italians for only four alleles. But it is generally believed that the first agriculturalists in the Near East were members of the same major race (Caucasoids) as the mesolithic hunter-gatherers of Europe. Using modern Turks or Ashkenazi Jews as representative Near Easterners, we note that neither group differs by as much as 0.2 from the Italians (compared for over 45 and 50 allele frequencies, respectively). Thus, we should not be surprised at the low number of alleles supporting the demic expansion hypothesis.

The highly significant negative partial correlation for the ABO system in Table 1 clearly contradicts the demic diffusion hypothesis. The well known east-west cline of the *B* allele of that system[25,26] runs perpendicularly to the spread of agricult (Fig. 1). Not surprisingly, it shows negative fit to the O hypothesis.

We conclude that the spread of agriculture through Europe was not simply a case of cultural diffusion, but involved significant differential reproduction of the new farmers whose origins can be traced to the Near East. Support for the hypothesis of Ammerman and Cavalli-Sforza is a necessary first step towards testing Renfrew's hypothesis of the origin of Indo-European speakers in Europe[27], which equates the early Indo-Europeans with the early farmers. We are currently examining the genetic evidence in favour of Renfrew's hypothesis. □

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Direct activation of cardiac pacemaker channels by intracellular cyclic AMP

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CYCLIC AMP acts as a second messenger in the modulation of several ion channels[1–9] that are typically controlled by a phosphorylation process[10]. In cardiac pacemaker cells, adrenaline and acetylcholine regulate the hyperpolarization-activated current (i_f), but in opposite ways; this current is involved in the generation and modulation of pacemaker activity[11]. These actions are mediated by cAMP and underlie control of spontaneous rate by neurotransmitters[12–17]. Whether the cAMP modulation of i_f is mediated by channel phosphorylation is, however, still unknown. Here we investigate the action of cAMP on i_f in excised patches of cardiac pacemaker cells and find that cAMP activates i_f by a mechanism independent of phosphorylation, involving a direct interaction with the channels at their cytoplasmic side. Cyclic AMP activates i_f by shifting its activation curve to more positive voltages, in agreement with whole-cell results. This is the first evidence of an ion channel whose gating is dually regulated by voltage and direct cAMP binding.

The current i_f was recorded in cell-free inside-out macro-patches excised from the membrane of isolated rabbit sino-atrial node myocytes and containing hundreds of i_f channels[18]. In Fig. 1*a* (left) it can be seen that i_f recorded in one such patch during hyperpolarizations to −95 mV increases during perfusion of protein kinase A (PKA; 100 U ml^{-1}) with cAMP (100 μM) at the intracellular side, in much the same way as i_f in whole-cell[14] and single-channel measurements[15] after β stimulation. By

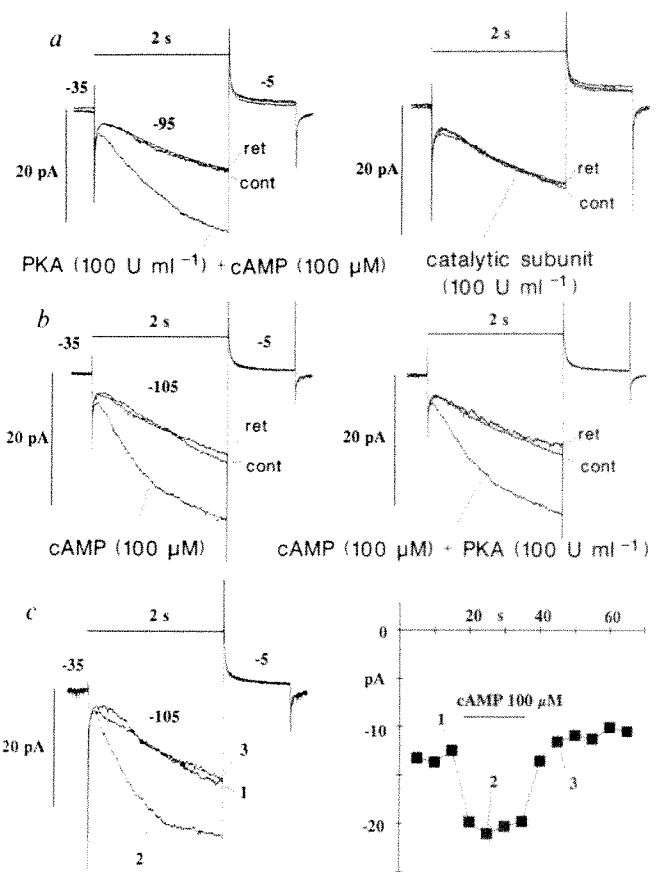

FIG. 1 Current i_f recorded in inside-out macro-patches. The current i_f was activated by stepping to the voltages indicated at a frequency of 0.2 Hz. All voltages are conventionally expressed as differences between internal and external solutions. In *a* and *b*, control and test solutions used for perfusion of the internal side of the patch contained ATP (2 mM) and Mg^{2+} (2 mM). In *c*, control and test solutions did not contain any ATP or Mg^{2+}. In *a* and *b*, each trace is the average of 2/3 recordings. *a*, Patch current records before (cont), during, and after (ret) exposure to a test solution containing protein kinase A (100 U ml^{-1}) plus cAMP (100 μM) (left) and, subsequently, before, during, and after perfusion with the catalytic subunit of the PKA (100 U ml^{-1}) (right). Assays run before and after the experiment showed that the kinase activity had been fully retained (1.3×10^6 U per mg protein just after purification), indicating that the lack of action of the catalytic subunit was not due to loss of enzyme activity; *b*, similar protocol in another patch perfused in sequence with test solutions containing cAMP at 100 μM (left) and cAMP (100 μM) plus PKA (100 U ml^{-1}) (right); *c*, action of cAMP in the absence of ATP. Left, patch i_f records before (1), during (2), and after (3) perfusion with cAMP at 100 μM; right, time-course of the i_f amplitude at −105 mV during cAMP perfusion.

METHODS. Single sino-atrial node myocytes were isolated from rabbit hearts as described[21]. Cells under study were allowed to settle in Petri dishes and superfused with a high-K^+ solution containing (mM): KCl, 130; NaCl, 10; $CaCl_2$, 2; EGTA, 5; HEPES–KOH, 10 (pH 7.4; pCa 7). Giga-seals were obtained with pipettes filled with a solution previously used for single i_f-channel recording[15] and containing (mM): NaCl, 70; KCl, 70; $CaCl_2$, 1.8; $MgCl_2$, 1; $BaCl_2$, 1; $MnCl_2$, 2; HEPES–KOH, 5; pH 7.4. To overcome the difficulty of detecting single i_f channel currents which results from their low conductance (0.98 pS)[15], large-tipped pipettes (about 1–3 μm diameter with resistances in the range 0.5–2 MΩ) were used which allowed recordings from macro-patches generating currents of several pA[18]. The number of channels in a patch was calculated to be up to ~1,000, and typically in the range 200/300, for the 111 patches considered here. After giga-seal formation, hyperpolarizations were applied in the cell-attached configuration from a holding potential of −35 mV (assuming a zero membrane-resting voltage in the high-K^+ solution used) to check for the presence of i_f channels. I_f records of several pA amplitude were frequently recorded under these conditions[18]. Patches giving successful recordings were pulled off the cell and perfused at the intracellular membrane side in the inside-out configuration, with control and test solutions delivered through a perfusion device that allowed sufficiently rapid (0.5–1 s) changes[21]. When patch excision led to vesicle formation, the pipette tip was briefly exposed to air to obtain the inside-out configuration. The perfusing control solution contained (mM): NaCl, 10; potassium aspartate, 130; $CaCl_2$, 2; EGTA, 5; HEPES–KOH, 10 (pH 7.2). ATP (Na^+ salt; 2 mM), $MgCl_2$ (2 mM) and GTP (0.1 mM) were present in some of the experiments. Temperature was continuously monitored and was 26–28 °C. ATP, GTP, PKA and PKA inhibitor were from Sigma; GDP-βS from Boehringer–Mannheim; catalytic subunit of PKA from Sigma, or purified in our laboratory from bovine heart[30]. Enzyme activity assayed after purification was 1.3×10^6 pmol min^{-1} mg^{-1} (1.3×10^6 U mg^{-1}), in agreement with previous values[30], and was abolished in the presence of the cAMP-dependent protein-kinase inhibitor (Sigma, type II); the purified enzyme ran as one band on an SDS-polyacrylamide gel with M_r ~40K.

analogy with other cardiac channels controlled by cAMP[3,7], this effect could be due to phosphorylation of the channel protein. Unexpectedly, however, activation of i_f was not associated with a phosphorylation process, as indicated in Fig. 1*a* (right), in which perfusion of the same patch with the catalytic subunit of PKA (100 U ml^{-1}) did not result in current activation. In 17 out of 22 patches, perfusion with the holoenzyme (100 U ml^{-1}) and cAMP (100 μM) resulted in i_f activation, whereas i_f activation was not observed in any of the 21 patches challenged with the catalytic subunit (activities in U ml^{-1}: 100, $n=4$; 500, $n=4$; 26,000, $n=7$; 36,400, $n=4$; 52,000, $n=2$). But perfusion with cAMP alone at 100 μM did stimulate the current i_f, as shown in Fig. 1*b* (left). The action of cAMP was not modified by addition of the holoenzyme (Fig. 1*b*, right), indicating that the i_f increase was attributable only to the cyclic nucleotide. We obtained i_f activation by cAMP in the presence of ATP in 26 patches. In 4 of these patches, PKA at 100 U ml^{-1} did not alter the i_f stimulation induced by 100 μM cAMP.

Activation of i_f by cAMP was not a result of phosphorylation by endogenous protein kinases, because it could be reproduced after removal of ATP and Mg^{2+} from the intracellular solution (Fig. 1*c*), and after addition of a non-specific protein-kinase inhibitor (H7 (1-(5-isoquinolinesulphonyl)-2-methylpiperazine; Sigma) at 10 μM, $n=4$; 100 μM, $n=2$) or of a specific PKA inhibitor (Sigma peptide inhibitor, rabbit sequence; 0.1 μM, $n=4$). The action of cAMP had a rapid onset (less than 5 s) and was also rapidly reversed (Fig. 1*c*, right). Stimulation of i_f by cAMP in the absence of ATP and Mg^{2+} was obtained in 61 out of 67 patches. Although phosphorylation-dependent steps may exist elsewhere in the modulation cascade[19], these data indicate that cAMP action is independent of phosphorylation.

It has been suggested that i_f channels may be directly coupled to G proteins[18]. We have excluded the possibility that cAMP action on i_f is mediated by G proteins, because it was still present in the absence of GTP ($n=24$) or when GTP was replaced by guanosine-5′-*O*-(2-thiodiphosphate) (GDP-β-S; 100 μM) as an inhibitor of G-protein function[20] ($n=5$).

In intact nodal cells increasing or decreasing intracellular cAMP does not alter the fully activated i_f, but shifts its activation curve to more positive or negative voltages[14,21]. So β stimulation increases the probability of single i_f channel opening, without changing the channel conductance[15]. Figure 2 shows that cAMP acts similarly on patch i_f. In Fig. 2*A*, cAMP at 100 μM increases i_f at −115 mV and decreases it at −155 mV, as expected for a positive shift of the activation curve[21], without modifying the fully activated current value at the latter voltage. In the more complete analysis of Fig. 2*B*, cAMP at 30 μM did not affect the fully activated *I*/*V* relation in the range −155/−25 mV (panel *c*), although it accelerated current activation and slowed current deactivation (panels *a* and *b*). Similar results were obtained in another cell. These results agree with the action of cAMP on i_f in whole-cell experiments[14,16,21].

Cyclic AMP-induced shifts of the i_f activation curve were measured[17] for different cAMP concentrations and the dose-response relationship is plotted in Fig. 3. Hill-plot fitting yielded values of 0.211 μM for the half-maximal cAMP concentration and 0.850 for the Hill coefficient. Cyclic AMP concentrations involved in the modulation of i_f gating are well within the range controlled under physiological conditions by adenylyl cyclase (0.2–5 μM in cardiac tissue)[22]. The maximal cAMP-induced shift of 11 mV is slightly larger than that caused by isoprenaline

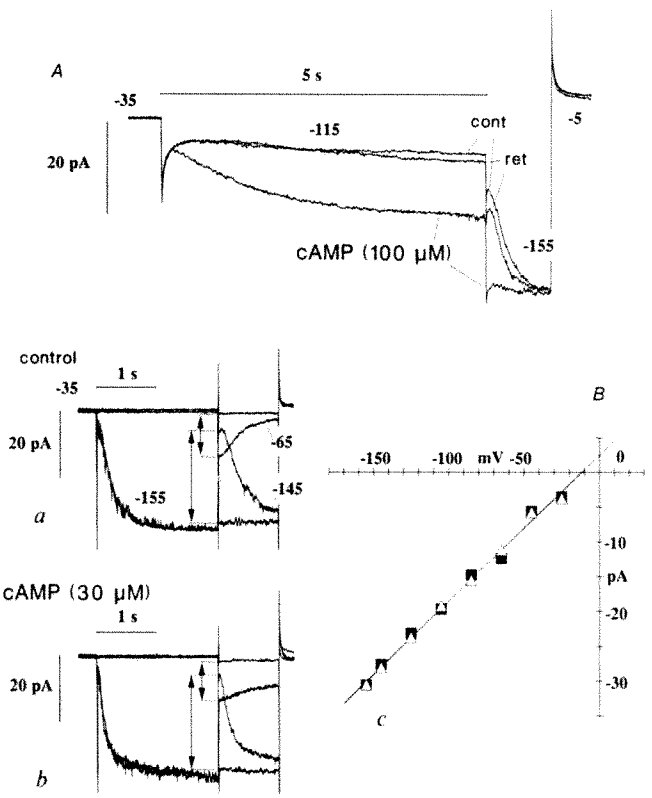

FIG. 2 *A*, Protocol applied every 10 s to an inside-our macro-patch before (cont), during, and after (ret) perfusion of the internal membrane side with cAMP at 100 μM. All solutions were ATP- and GTP-free. In the control solution, stepping to −115 mV activated a fraction of the available i_f channels, and the following step to −155 mV activated the remaining fraction. During perfusion with cAMP, the fraction of current activated at −115 mV increased, while that at −155 mV decreased. This indicates that cAMP displaced the i_f activation curve to more positive voltages without alteration of the fully activated current at −155 mV. *B*, Lack of action of cAMP on the i_f fully activated *I*/*V* relation. *a* and *b*, Patch i_f records in a control (ATP- and GTP-free) solution (*a*) and after addition of cAMP at 30 μM (*b*). The procedure has been described[14] and consisted of paired clamp protocols in which steps to test voltages were either delivered from the holding potential of −35 mV, or were preceded by a fully activating pulse to −155 mV for 2 s. The *I*/*V* curve was then measured as the amplitude of the difference current at each test potential (arrows). Paired records at −65 and −145 mV are shown in both control (*a*) and cAMP (*b*) solutions. All traces were digitally corrected for linear leakage and capacitative transients. *c*, Fully activated *I*/*V* relation for patch i_f in the absence (■) and in the presence (△) of cAMP at 30 μM. Best-fitting linear relations (full lines) have reversal potentials of −11.5 mV (control) and −12.4 mV (cAMP) and slope conductances of 209 pS (control) and 211 pS (cAMP). From these values and the known single i_f channel conductance[15], the channels in this patch were about 213–215.

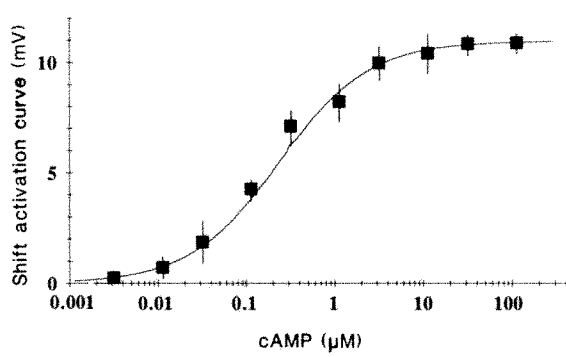

FIG. 3 Dose-response relationship for the shift of i_f activation curve as a function of cAMP concentration from 35 patches. Shifts were measured as described[17]. Mean ± s.e.m. values are plotted. The numbers of patches tested at the various concentrations (μM) were: 4 (0.003), 4 (0.01), 4 (0.03), 4 (0.1), 8 (0.3), 7 (1), 6 (3), 6(10), 9 (30) and 13 (100). Data were best fitted using the Hill equation $y/y_{max}=1/(1+(k_{1/2}/c)^n)$, where y is the measured shift, y_{max} is the shift at saturating concentrations (set to 11 mV) and c is the cAMP concentration (full line). This yielded a half-maximal concentration $k_{1/2}=0.211$ μM and a Hill factor $n=0.850$.

(1 μM) in whole cells (7.84 ± 0.72 mV; $n = 11$), as is to be expected if a basal level of cAMP is present in sino-atrial node myocytes[16].

The i_f was also activated, although with a reduced specificity, by cGMP and cCMP, which we tested for comparison with other cyclic nucleotide-sensitive conductances in receptor cells[23-26]. We measured shifts of 1.10 ± 0.66 ($n = 4$), 5.27 ± 0.08 ($n = 3$) and 9.63 ± 0.49 ($n = 8$) mV with cGMP at 0.3, 10 and 100 μM, respectively; and 2.59 ± 0.47 ($n = 14$), 5.87 ± 0.41 ($n = 17$) and 6.24 ± 0.98 ($n = 18$) mV with cCMP at 10, 100 and 1,000 μM, respectively. Apparent dissociation constants and Hill coefficients, assuming maximal shifts of 11 mV (for cGMP) and 6.5 mV (for cCMP), were $k_{1/2} = 7.85$ μM and $n = 0.704$ (cGMP), and $k_{1/2} = 11.85$ μM and $n = 0.784$ (cCMP).

By modulating i_f and other (K^+ and Ca^{2+}) currents, cAMP plays an essential part in the control of electrical and contractile activity of cardiac myocytes. Although cardiac Ca^{2+} and K^+ channels[3,7], as well as several other channels in different tissues[10], are modulated by cAMP by phosphorylation, our data indicate that the action of cAMP on i_f is exerted by direct interaction with the i_f channels.

Direct channel regulation by cyclic nucleotides has been demonstrated in various receptor cells[23-26], of which only olfactory receptors are sensitive to cAMP[26]. Although, interestingly, in all cases non-specific cation conductances like i_f are controlled by cyclic nucleotides, the i_f modulation by cAMP reported here is different in that (1) i_f channels are voltage-gated; (2) sino-atrial node cells are non-receptor cells; (3) i_f channels are more sensitive to cAMP than to cGMP; (4) the Hill coefficient of i_f modulation by cAMP (0.824) is lower than that in sensory channels[26,27] and suggests a 1:1 binding.

I_f channel modulation by cAMP in the sino-atrial mode cell underlies neurotransmitter control of the cell spontaneous activity and of cardiac rhythm. The presence of a direct, phosphorylation-independent control of i_f channel gating provides a rapid and efficient mode of channel control not requiring metabolic input.

Several types of nerve cells possess i_f-like currents[11], for some of which cAMP-dependent stimulation has been reported[28,29]. It will be interesting to know if cAMP directly regulates i_f-like currents in these preparations also. □

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Immunomodulation of experimental allergic encephalomyelitis by antibodies to the antigen–Ia complex

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AUTOIMMUNE diseases occur when T lymphocytes become activated on recognizing self antigen linked to the autologous class II molecule of the major histocompatibility complex (MHC)[1,2]. The resulting complex of antigen MHC T-cell receptor could be a target for treatment of autoimmune diseases. Studies in which each component is blocked separately[3-10] might be limited by interference in non-relevant immune responses that either use the same set of T-cell-receptor *V* gene segments or are linked to the same MHC. We report here an attack by a specific antibody on the unique antigenic site formed by the binding of two components of the trimolecular complex, the autoantigen bound to the self MHC[11-14]. We tested its effect in experimental allergic encephalomyelitis, an acute neurological autoimmune disease which is widely regarded as a model for autoimmune disorders[15-17] and which is mediated by $CD4^+$ T cells recognizing myelin basic protein (BP), or its peptides, in association with self Ia[18,19]. We made monoclonal antibodies which bound only the complex of BP and $I\text{-}A^s$. These antibodies blocked the proliferative response *in vitro* to the encephalitogenic determinant of BP and reduced the response to intact BP, without affecting the response to a nonrelevant antigen–purified protein derivative of tuberculin presented on syngeneic macrophages. They also inhibited experimental allergic encephalomyelitis in $H\text{-}2^s$ mice. Hence, antibodies directed specifically to the autoantigen–Ia complex, may offer a highly selective and effective treatment in autoimmune diseases.

To obtain anti-BP/Ia^s monoclonal antibodies, $B_{10}D_2$ mice were immunized with glutaraldehyde-fixed SJL/J macrophages that had been pulsed with rat basic protein (RBP) and their spleen cells fused with the NSo plasmacytoma line. The hybridomas obtained were screened for their ability to block selectively the proliferation of three different effector T-cell clones specific to: (1) the same antigen and the same Ia of the immunizing cells (SJL-RBP-6); (2) another antigen-purified protein derivative of tuberculin on the same *H-2* haplotype (SJL-PPD-5); and (3) RBP on a different *H-2* haplotype (B_{10}PL-RBP-20). The results for representative hybridomas are summarized in Table 1. Out of 800 hybridomas tested, 778 had no effect, as represented by clone C-25; five lines (for example A-6) inhibited the responses of all the clones tested non-specifically; seven hybridomas, represented by C-11, were specific to Ia^s and inhibited only the $H\text{-}2^s$ T-cell clones; and six other lines (for example B-10) recognized the antigen BP and therefore inhibited all BP-specific clones. Only four hybridomas, (A-10-12, B-7-1, B-18-7 and C-34-72) inhibited exclusively the proliferative response of the SJL-RBP-6 encephalitogenic clone, with little or no effect on the two non-relevant clones. These antibodies were tested for specificity to the BP/$H\text{-}2^s$ complex.

The ability of these antibodies (all IgM by isotype analysis), to bind specifically to SJL/J peritoneal macrophages pulsed with RBP was demonstrated both by flourescence-activated cell sorting (FACS) analysis and complement-mediated cytotoxicity assay. FACS analysis showed that 44.3 ± 3.0% and 43.2 ± 2.8%

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of RBP-pulsed SJL/J macrophages were stained by antibodies B-18-7 and C-34-72, respectively, in comparison with 32.6 ± 0.1% and 30.3 ± 0.7% binding to unpulsed macrophages. Only 29.4 ± 0.9% of the RBP-pulsed SJL/J cells were stained by non-relevant IgM control antibody (anti ricin). There was similar staining, 30.4 ± 1.1% and 32.1 ± 0.6%, with RBP-pulsed macrophages of BALB/c (H-2^d) mice in the presence of B-18-7 and C-34-72 antibodies, respectively. Thus 10–15% specific binding has been demonstrated. These results were reproducible in three independent experiments using either culture supernatant or ascitic fluid. In competition experiments (data not shown) the B-18-7 and C-34-72 antibodies consistently inhibited, though to a very limited degree (about 3%), the binding of anti-I-A^s serum to RBP-pulsed macrophages. This may be because only a small proportion of I-A molecules are occupied by the processed antigen[21], or because of the low affinity of these antibodies in comparison with the anti-I-A^s serum. A functional assay of complement-mediated cytotoxicity showed (Fig. 1*A*) specific lysis by antibodies B-7-1, B18-7 and C-34-72 only when RBP or peptide 89–101 (the relevant encephalitogenic determinant in H-2^s mice[22,23]) were presented on SJL/J but not on BALB/c macrophages. These antibodies did not lyse a non-relevant antigen, lysozyme, even when presented on SJL/J haplotype, nor did they lyse untreated SJL/J macrophages. The results (11.4–15.9% specific lysis) were comparable with those from the FACS analysis, and to other published results[21]. These antibodies did not bind to free RBP or to peptide 89–101 in a solid-phase radioimmunoassay (data not shown). These data indicate that these antibodies recognize only the 89–101/Ia^s complex.

We then tested the effect of the anti-BP/Ia^s antibodies on the T-cell response of mice that had been treated with the antibodies after immunization with RBP (Fig. 1*B*). The antibodies completely blocked the T-cell response of the SJL/J mice to the encephalitogenic determinant, and reduced the response to the intact BP from either rat (RBP) or mouse (MBP) (Fig. 1*B*, *a*). This inhibition was specific, as the response to PPD was not effected. The anti-complex antibodies inhibited only the response of H-2^s mice, SJL/J and $B_{10}S$ (Fig. 1*B*, *a* and *B*, *b*), but no inhibition was observed in SWR (H-2^q) and $B_{10}PL$ (H-2^u) mice (Fig. 1*B*, *e* and *B*, *d*). The humoral response of H-2^s mice

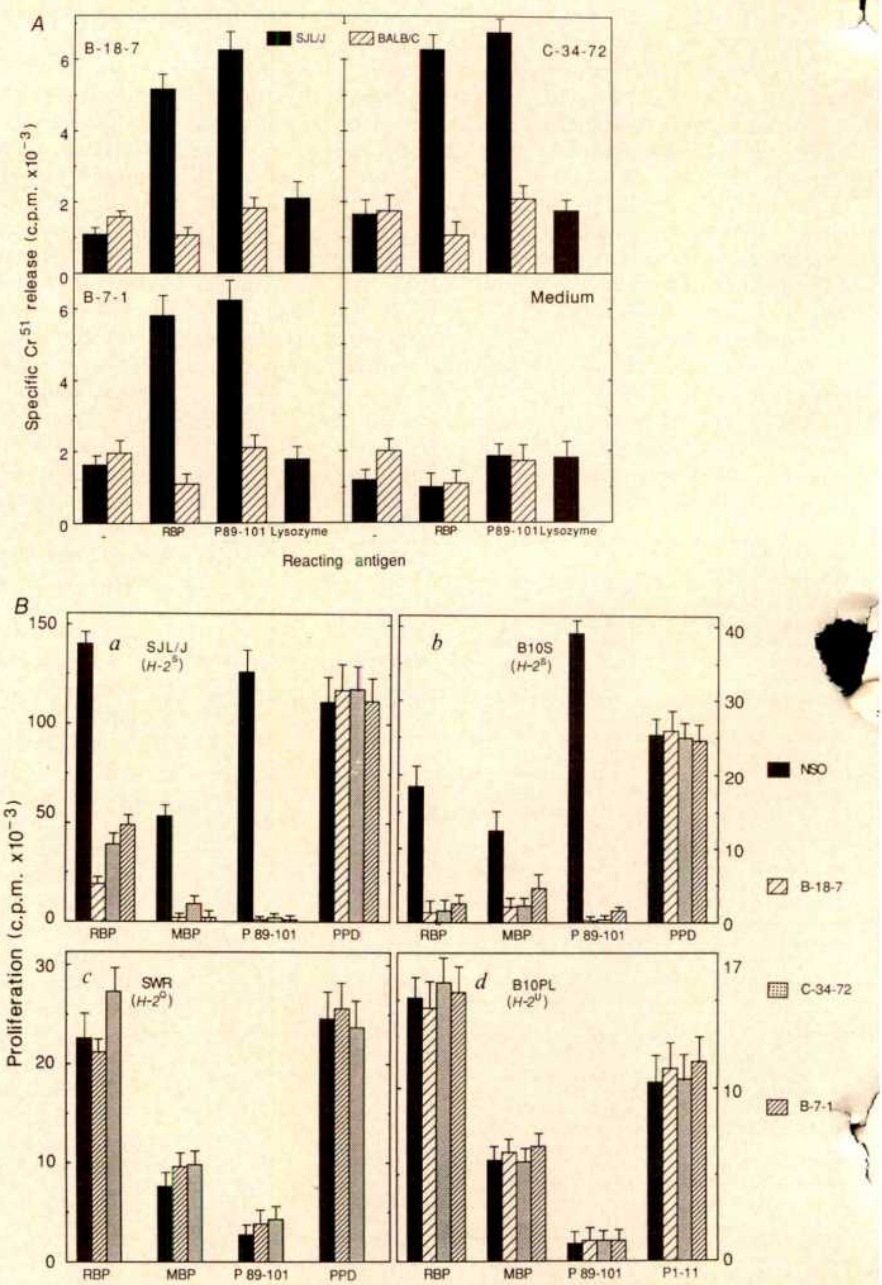

FIG. 1 *A*, Specificity of monoclonal antibodies to antigen/Ia complex, complement mediated cytotoxicity on antigen-pulsed macrophages. *B*, Anti-antigen/Ia effect on the T-cell response of mice from various strains immunized with RBP. METHODS. *A*, Peritoneal macrophages (1×10^6 cells per ml), were cultured for 18 h in medium supplemented with 200 units ml^{-1} murine interferon-γ and 100 μCi ml^{-1} $^{51}CrO_4$. During the last 2 h of culture, cells were pulsed with either RBP (10 mg ml^{-1}), peptide 89–101 (1 mg ml^{-1}) or lysozyme (10 mg ml^{-1}). The target cells were then incubated with hybridoma supernatants (50 μl) and 1:10 diluted rabbit complement (from fresh serum of young rabbit). After 1 h supernatant samples were collected and ^{51}Cr release was determined. The experiment was repeated four times. The bar graphs plot the mean c.p.m. of triplicate samples after subtraction of the spontaneous release obtained by mixing target cells with media alone for the duration of the assay (327 ± 31). Error bars indicate standard deviation. Maximal release after total Nonidet P-40 lysis was 37,109 ± 4,295 and 30,737 ± 3,957 for SJL/J and BALB/c, respectively. The specific lysis obtained by the anti-complex antibodies was 11.4–15.9%. These values were calculated as follows: % specific lysis = [(experimental − unpulsed control)/(total − unpulsed control)] × 100. *B*, Mice were immunized with RBP (400 μg per mouse) on day 0, in complete Freund's adjuvant supplemented with 4 mg ml^{-1} H37Ra mycobacterium tuberculosis, followed by i.v. injections of pertussis toxin immediately and 48 h later. Ascitic fluids (0.5 ml), of anti-antigen/Ia antibodies, or NSo ascities which contained predominantly IgM isotype, were injected into the peritoneum on days −2, −1, 0, +1, +2, +3, +5, +7, +9. Lymph nodes and spleen cells were taken after 10 days and tested in proliferation assay, using 5×10^5 cells and the following antigens: RBP (10 μg), MBP (10 μg), P89-101 (1 μg), P1-11 (1 μg), for 4 days. The experiment was performed four times. Results are expressed as the mean c.p.m. of triplicates ± s.d. after subtracting the thymidine incorporation of cells incubated without antigen.

TABLE 1 Inhibition of T-cell proliferation by representative monoclonal antibodies

Inhibitor	Proliferation, Δ c.p.m. (inhibition, %) SJL-RBP-6		SJL-PPD-5		B10.PL-RBP-20	
Medium	28,752	(0%)	26,196	(0%)	53,776	(0%)
C-25	29,951	(0%)	26,003	(1%)	54,169	(0%)
A-6	3,165	(89%)	8,648	(66%)	11,813	(78%)
C-11	17,629	(39%)	17,831	(32%)	52,172	(3%)
B-10	12,078	(58%)	24,309	(7%)	19,143	(64%)
A-10-12	15,483	(46%)	24,404	(7%)	51,949	(4%)
B-7-1	521	(98%)	26,254	(0%)	44,257	(18%)
B-18-7	132	(100%)	25,304	(4%)	51,483	(4%)
C-34-72	764	(97%)	27,001	(0%)	48,401	(10%)

BP-Ia^s complexes for immunization were prepared by *in vitro* pulsing of SJL/J spleen cells with 10 μg ml^{-1} RBP for 8 h. RBP had been prepared as described previously[23]. Cells were then washed three times and fixed with 1% glutaraldehyde. The antigen-pulsed spleen cells were injected in complete Freund's adjuvant to the footpads of $B_{10}D_2$ mice (100×10^6 per mouse). Three more intraperitoneal injections of pulsed macrophages in PBS were given at two-week intervals. Four days after the last injection, spleen cells from immunized mice were fused with NSo plasmacytoma cells in a ratio of 4:1, respectively, according to Eshhar *et al.*[24]. Screening was performed by adding 50 μl of hybridoma culture supernatants to 2.5×10^4 T cells, 5×10^5 irradiated spleen cells and 2.5 μg per well of antigen. After 48 h, the cultures were pulsed with [^{3}H]thymidine and cells were collected 16 h later. The mean c.p.m. [^{3}H] thymidine incorporation was calculated for triplicate cultures. Standard deviations for triplicate cultures were within 20% of the mean. Incorporation of the controls without antigen was less than 1,000 c.p.m.

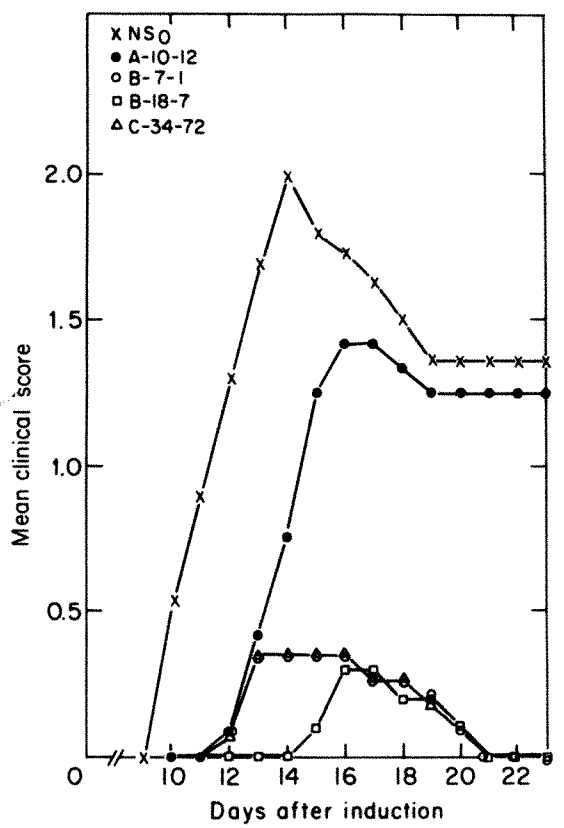

FIG. 2 Inhibition of EAE by anti-antigen/Ia complex antibodies in SJL/J mice. METHODS. EAE was induced on day 0, by injecting 5 mg lyophilized mouse spinal cord homogenate in enriched CFA followed by pertussis toxin as decribed in Fig. 1*B*. Ascitic fluids of monoclonal antibodies (0.5 ml) were injected into the peritoneum on days −2, −1, 0, +1, +2, +3, +5, +7, +9. After 10 days mice were examined daily for signs of EAE and assessed for clinical severity as follows: 1, flaccid tail; 2, hind limbs paralysis; 3, hind and fore limbs paralysis; 4, total paralysis; 5, moribund. Each point represents daily mean clinical score of 10–12 mice.

TABLE 2 Inhibition of EAE by anti-antigen/Ia complex antibodies in mice of various strains

Strain of mouse	NS_0 Incidence of disease Clin.	Hist.	Clinical score 0–5	B-18-7 Incidence of disease Clin.	Hist.	Clinical score 0–5
SJL/J	8/11 (73%)	5/6 (83%)	2.2	1/10 (10%)	0/5 (0%)	0.3
H-2^s	2/7* (28%)	5/7* (71%)	0.3*	0/10* (0%)	0/10* (0%)	0*
ASW H-2^s	3/7 (42%)	3/7 (42%)	0.6	0/13 (0%)	0/13 (0%)	0
SWR H-2^q	7/13 (53%)	5/6 (69%)	1.1	9/13 (83%)	5/6 (83%)	2.5
B_{10}PL H-2^u	5/10 (50%)	4/6 (66%)	0.7	5/10 (50%)	4/6 (66%)	0.9

EAE was induced on day 0 by immunization with either spinal cord homogenate, as described in Fig. 2, or by RBP, 400 μg per mouse (entries indicated by an asterisk). Inhibition was obtained by intraperitoneal injection of 0.5 ml of the tested ascites on days −2, −1, 0, +1, +2, +3, +5, +7, +9. Clin., clinical; Hist., histological.

was also inhibited by the anti-complex antibodies (data not shown). The anti-BP/Ia^s antibodies reduced antibody formation to BP, and removed the response to peptide 89–101, but had no effect on the antibody response to PPD. These findings are indicative of the role of the T helper cells, which also recognize the antigen when bound to Ia, in the antibody response to BP.

The anti-BP/Ia^s antibodies were also tested for their capacity to block the clinical and histological manifestations of experimental allergic encephalomyelitis (EAE) (Fig. 2). Three clones, B-7-1, B-18-7 and C-34-72, caused inhibition of the disease in SJL/J mice in comparison with control ascitic fluid of the NSo plasmacytoma line. The hybridoma A-10-12, which was less active *in vitro* (Table 1), was also less efficient in the inhibition of EAE induction *in vivo*. The inhibition was specific to the *H-2^s* haplotype as it was also observed in ASW mice (Table 2), whereas in other EAE-sensitive strains of different haplotype, for example in SWR (*H-2^q*) and B_{10}PL (*H-2^u*) there was no effect. The anti-BP/Ia^s antibodies inhibited EAE induced by both spinal cord homogenate and purified BP.

These results suggest that by immunizing against the autoantigen presented by self MHC, specific anti-complex antibodies that bind only to the specific antigen in the right MHC context can be obtained. Such antibodies suppress specifically the pathogenic process and not other T-cell responses linked to the same MHC. These antibodies do not exert an adverse effect on myelin, as they cannot recognize the MBP in the target organ and thus offer a highly selective and effective treatment to EAE. This approach might be applied to the human analogue of EAE, namely multiple sclerosis, and to other autoimmune diseases. Even though macrophages pulsed with the whole BP were used for immunization, antibodies which specifically recognize the encephalitogenic peptide 89–101 presented on the Ia^s were selected by using a pathogenic T-cell clone for screening. Thus, it is not essential to identify the disease-inducing determinant in order to apply this strategy in other systems where exact knowledge of the autoantigen is not yet available. □

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Clonal deletion of immature $CD4^+8^+$ thymocytes in suspension culture by extrathymic antigen-presenting cells

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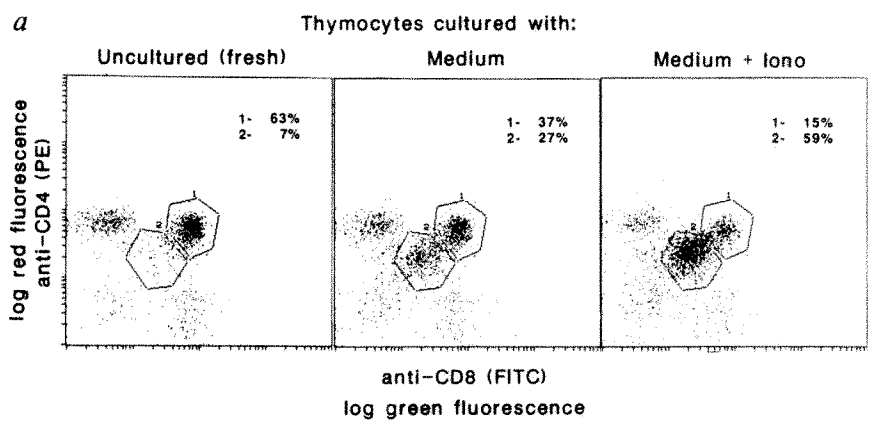
ONE mechanism ensuring self tolerance of T cells is the clonal deletion of thymocytes bearing $\alpha\beta$ T-cell receptors[1–4]. The stage of thymocyte development at which the interaction with antigen-presenting cells (APCs) leads to deletion, however, has not been determined directly. Indirect evidence suggests that intrathymic APCs induce deletion of $CD4^+8^+$ thymocytes[3–6] (which die by apoptosis[7]) but deletion at less[8] and more mature[9] developmental stages has also been implied. It is also not clear if clonal elimination of thymocytes can be triggered by peripheral antigens carried on extrathymic APCs migrating through the thymus[10]. Here we show antigen-specific induction of apoptosis in $CD4^+8^+$ thymocytes cultured in suspension, by thymic as well as splenic APCs. Thus the recognition of antigen by $CD4^+8^+$ thymocytes may lead to deletion, suggesting that this is the central mechanism of tolerance induction, which is not limited by the antigen-presenting ability of the thymic stroma.

When thymocytes are cultured in single-cell suspension, a distinct population of cells appears which express reduced levels of CD4 and CD8 molecules. This population represents immature $CD4^+8^+$ thymocytes 'spontaneously' undergoing programmed cell death (apoptosis), which can be accelerated by known inducers such as ionomycin (Fig. 1 and ref. 11). To find out if this observation could be used to study the mechanism of antigen-specific induction of apoptosis in immature T cells, we used thymocytes from $\alpha\beta$ T-cell receptor (TCR) transgenic mice expressing, on more than 90% of $CD4^+8^+$ thymocytes, a TCR specific for the male (H-Y) antigen presented by class I major histocompatability complex (MHC) molecules ($H\text{-}2D^b$)[3].



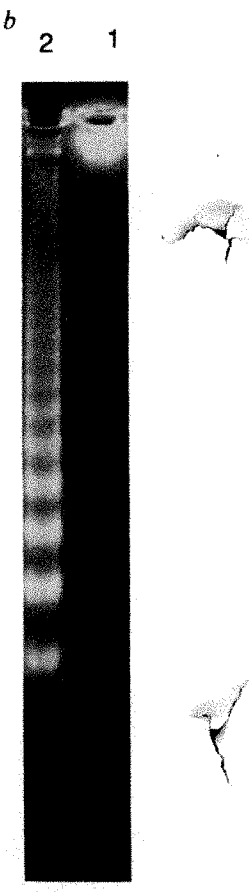


FIG. 1 Identification of $CD4^+8^+$ thymocytes undergoing apoptosis in suspension culture. METHODS. *a*, Thymocytes (2×10^6 ml^{-1}) from normal B6 mice were cultured for 24 h (37°C, 5% CO_2) in 24-well Costar tissue culture plates, in Iscove's modified Dulbecco's medium supplemented with 2×10^{-5} M 2-mercaptoethanol, penicillin (100 U ml^{-1}), streptomycin (100 μg ml^{-1}) and 10% FCS without or with ionomycin (Iono, 5 μg ml^{-1}). The surface expression of CD4 and CD8 molecules on cell populations (from which only cell debris but not dead cells were excluded by forward and side-scatter gating) was analysed before and after *in vitro* culture by direct staining with the mixture of phycoerythrin (PE)-labelled anti-CD4 and FITC-labelled anti-CD8 monoclonal antibodies (Becton and Dickinson) as previously described[3]. Two populations of $CD4^+8^+$ thymocytes expressing high (gate 1) and low (gate 2) levels of CD4/CD8 molecules are shown. *b*, CD4/CD8 'high' and 'low' populations were sorted out by fluorescence-activated cell sorting, their DNA was isolated and analysed by agarose gel electrophoresis as described[18]. Lanes contained DNA isolated from CD4/CD8 'high' cells (lane 1) and 'low' cells (lane 2). Note that DNA in lane 2 is degraded into oligonucleosomal fragments, which is the characteristic feature of apoptosis, whereas DNA in lane 1 is not.

Thymocytes from H-2^b transgenic females were cultured with thymic or splenic adherent cells from B6 (H-2^b) males or females for different times, then collected and analysed for surface expression of CD4 and CD8 molecules. In control cultures, female transgenic thymocytes were cultured with male adherent cells from BALB/c (H-2^d) or AKR (H-2^k) mice and thymocytes from nontransgenic B6 female mice were cultured with male or female adherent cells (Fig. 2). During 24–48 h of culture, deletion of $CD4^+8^+$ thymocytes occured only in the presence of cells bearing H-Y antigen with the appropriate (H-2^b) MHC molecules. During the first 12 h of coculture of transgenic thymocytes with H-2^b male APCs, more than 50% of $CD4^+8^+$ thymocytes underwent apoptosis as shown by decreased expression of CD4 and CD8 molecules on $CD4^+8^+$ thymocytes, the proportion of which decreased only slightly in that time. After 48 h very few $CD4^+8^+$ thymocytes expressed normal levels of CD4 and CD8 molecules and the proportion of all $CD4^+8^+$ cells was much reduced. That apoptotic cells did not accumulate in culture later on, is probably due to their removal by macrophages in the APC preparations as well as to desintegration of cells.

These changes are observed only with APCs expressing the appropriate antigen. Other APCs that express either the wrong MHC antigen or lack the male antigen do not seem to induce apoptosis in $CD4^+8^+$ thymocytes, as there was only a moderate decrease of the proportion of $CD4^+8^+$ cells expressing normal levels of CD4 and CD8 molecules; and the proportion of cells with reduced expression of CD4 and CD8 molecules did not significantly differ from the proportion of cells undergoing 'spontaneous' apoptosis at the beginning of culture. Similar results were obtained when transgenic thymocytes were cocultured with thymic accessory cells (data not shown, but see below). These experiments did not rule out the possibility that the deletion of $CD4^+8^+$ thymocytes is the consequence of antigen recognition by mature thymocytes present in suspension, which could release nonspecific factors toxic for immature thymocytes. This possibility was suggested by our observation that during ontogeny, deletion of $CD4^+8^+$ thymocytes in transgenic males

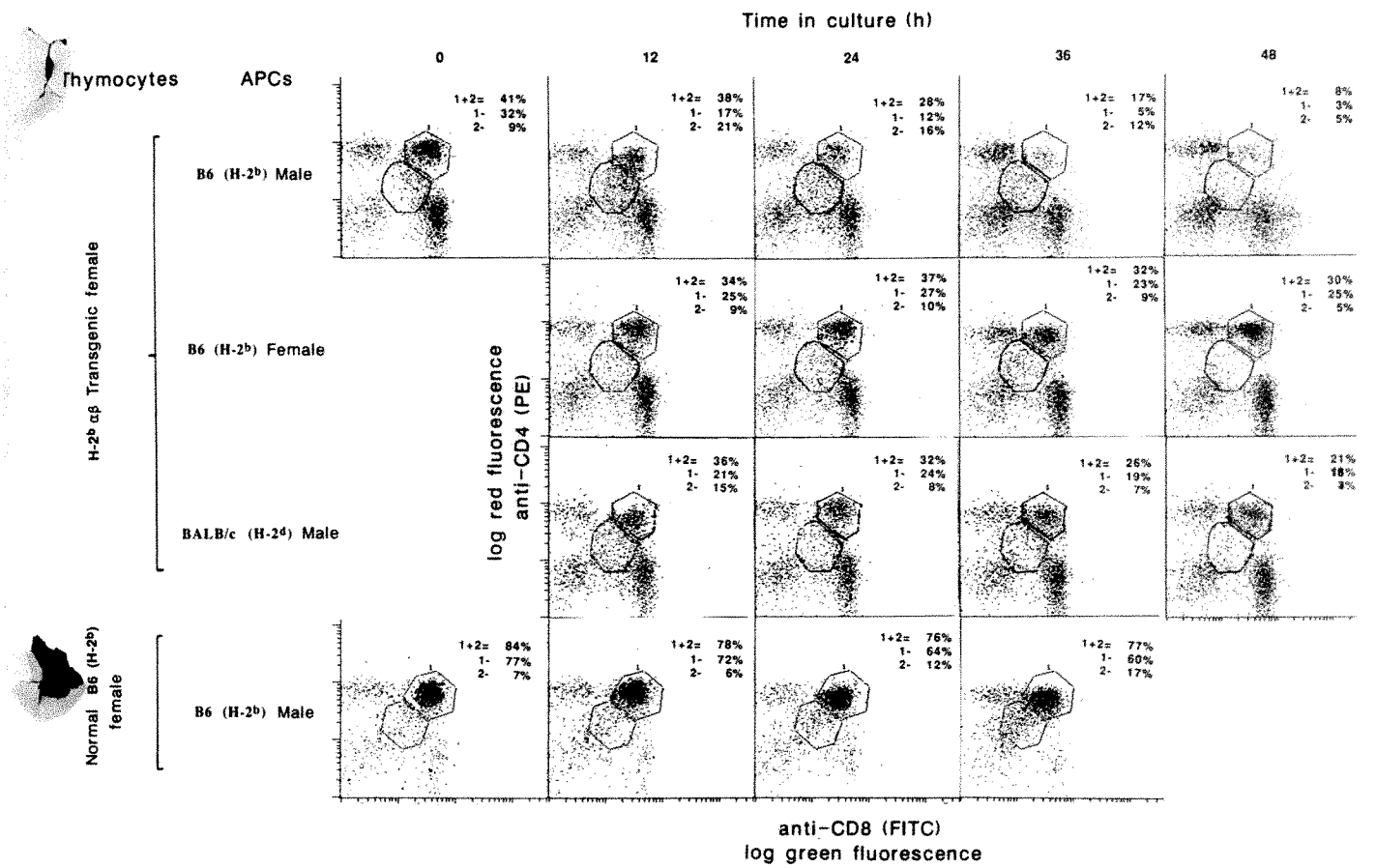


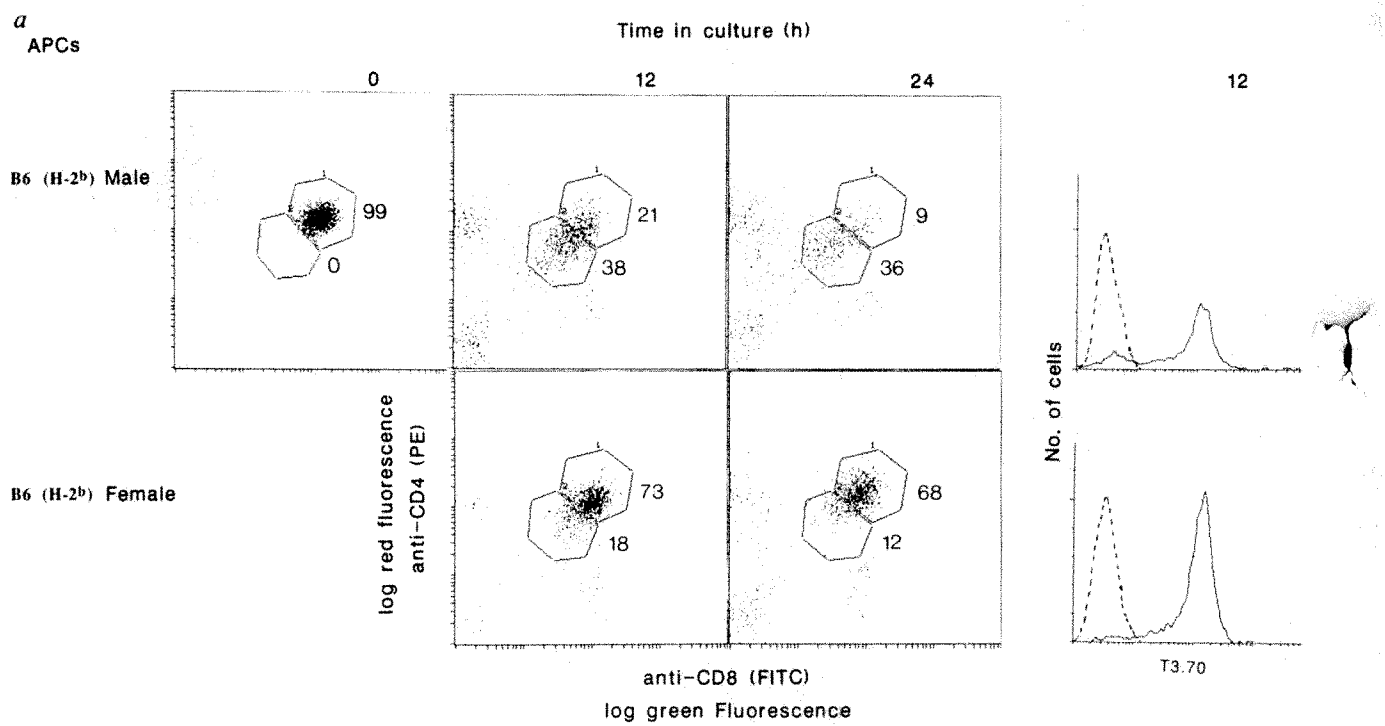
FIG. 2 *In vitro* antigen-specific induction of apoptosis of $CD4^+8^+$ thymocytes expressing transgenic TCR specific for male (H-Y) antigen in the context of H-2D^b molecules. Thymocytes from H-2^b transgenic or normal female mice were cultured for the indicated period of time on the monolayers of adherent APCs, prepared from spleens of male or female mice of different H-2 haplotypes. Thymocytes were collected and analysed for expression of CD4/CD8 molecules. Similar results were obtained when thymic APCs were used instead of splenic APCs. There was no marked clonal deletion when APCs from male AKR (H-2^k) mice were cocultured with transgenic female thymocytes (not shown). In different experiments, in all control groups, the total numbers of thymocytes recovered after 48 h varied from 50–70% of the input with 5–15% fluctuations between them. The recovery of female transgenic thymocytes cocultured with B6 male APCs was 30–50% lower than in control groups.

METHODS. Monolayers of adherent thymic and splenic APCs were prepared as follows. Removed organs were placed in cold IMDM, cut into small (~1 mm^3) fragments and washed free of the released cells by several rounds of resuspending, unit gravity sedimentation, and discarding cells floating in the supernatant. The remaining tissue fragments were transferred into 16-mm wells of 24-well Costar tissue culture plates (10–20 fragments per well) and cultured in IMDM supplemented with 20% FCS (37 °C, 5% CO_2). During the first week, cultures were fed with fresh medium every 2 days by exchanging 50% of medium and then every second or third day washed to remove nonadherent cells. Thymocytes (2×10^6 ml^{-1}) were added to these primary explants of adherent cell cultures when they reached ~75% confluency (one to three weeks after initiation of cultures). Similar results were obtained with freshly isolated splenic or thymic adherent APCs prepared by 2-h incubation (37 °C, 5% CO^2) of cell suspensions on Petri dishes followed by washing out of nonadherent cells and transfer of counted numbers of adherent cells (2×10^6 per well) which were collected by incubation with trypsin-EDTA solution (Gibco). The CD4/CD8 phenotype of cultured thymocytes was analysed as previously described[3]. The preparations of adherent cells from spleen and thymus contained 40% and 20–30% of $Mac1^+$ cells, respectively, less than 5% of $CD4^+$, less than 5% of $CD8^+$ and >95% class I MHC^+ cells (data not shown).

was undetectable before day 18 of gestation[12], coinciding with the appearance of more mature subpopulations of thymocytes[13].

To test whether apoptosis of $CD4^+8^+$ thymocytes resulted from a direct interaction with APCs, we separated $CD4^+8^+$ thymocytes from other thymocyte subpopulations by cell sorting (purity > 99%) and cocultured them with thymic or splenic APCs from B6 males or females. The removal of the mature thymocyte populations did not affect the ability of male thymic APCs to induce antigen-specific apoptosis of $CD4^+8^+$ thymocytes expressing the transgenic TCR (Fig. 3). Unlike previous experiments, which were compatible with tolerance being due to an arrest of development[3–6,14], this result indicates that $CD4^+8^+$ thymocytes can be the target of antigen-specific deletion.

The ability of thymic as well as extrathymic APCs to induce



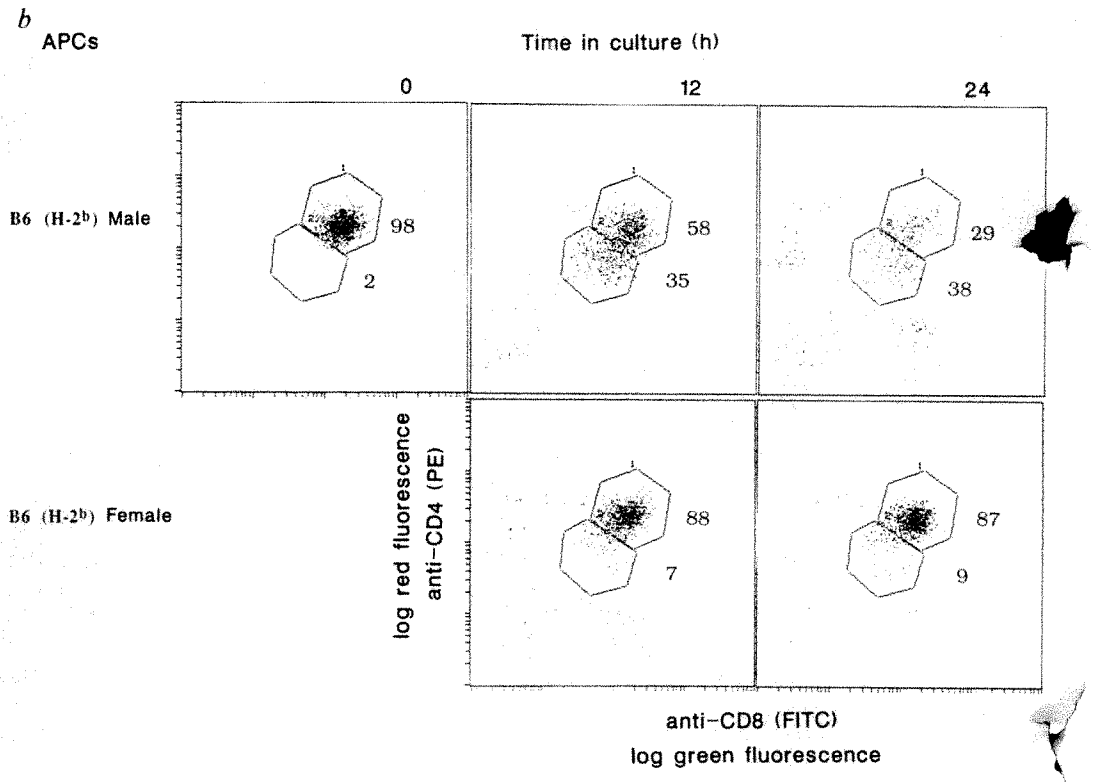


FIG. 3 Deletion by apoptosis of male antigen-specific $CD4^+8^+$ thymocytes results from direct antigen-specific interaction with APCs. The $CD4^+8^+$ thymocytes purified by cell sorting (>99% pure) were cultured on monolayers of thymic (*a*) or splenic (*b*) APCs and analysed for surface expression levels of CD4, CD8 and transgenic TCR molecules. Numbers indicate the proportions of viable $CD4^{high}8^{high}$ (gate 1) and 'detectable' apoptotic $CD4^{low}8^{low}$ (gate 2) cells. METHODS. For detection of transgenic TCR expression, indirect staining with T3.70 monoclonal antibody (solid line), specific for transgenic alpha chain, was performed as previously described[11]. Control staining with second-step reagent (FITC-labelled goat anti-mouse Ig) is indicated by broken line. The recovery of $CD4^+8^+$ thymocytes cultured on female or male APCs were 70% and 35% after 12 h and 45% and 10% after 24 h, respectively (*a*) and 80% and 50% after 12 h and 65% and 20% after 24 h, respectively (*b*). The $CD4^+8^-$, $CD4^-8^+$ and $CD4^-8^-$ cells, which can be seen on the dot plots, represent contaminating, nontransgenic lymphocytes surviving the procedure of preparation of APCs. That they are of nontransgenic origin is indicated by the fact that the expression level of the transgenic TCR (T3.70) seen in *a* shows normal distribution, characteristic for the majority of $CD4^+8^+$ thymocytes[12].

antigen-specific deletion of $CD4^+8^+$ thymocytes indicates that it is not a unique property of the thymic microenvironment, but is the property of a certain developmental stage of T cells which makes it sensitive to deletion. This agrees with the recent study in thymic organ cultures from normal mice, in which the ability of peripheral dendritic cells to induce unresponsiveness in developing T cells by an unknown mechanism was shown[15]. Thus, antigens carried on APCs from the periphery into the thymus may induce central tolerance. The demonstration of antigen-specific deletion of $CD4^+8^+$ thymocytes in suspension culture offers new possibilities in the study of the selection mechanisms operating at this critical stage of T-cell development[16]. In particular, the role of various T-cell receptors and their ligands (for example, TCR, CD4, CD8, antigenic peptides[17] and MHC molecules), as well as the role of different stromal cells, in determining whether deletion (apoptosis) or positive selection (inhibition of apoptosis) will result from interaction of immature thymocytes with thymic microenvironment, may be directly approached in a defined *in vitro* system. □

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Parental imprinting of the mouse H19 gene

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THE mouse *H19* gene encodes one of the most abundant RNAs in the developing mouse embryo[1]. It is expressed at the blastocyst stage of development, and accumulates to high levels in tissues of endodermal and mesodermal origin (H. Kim, unpublished result). After birth the gene is repressed in all tissues except skeletal muscle. It lacks a common open reading frame in the 2.5-kilobase RNA, but has considerable nucleotide sequence similarity between the genes of rodents and humans[2,3]. Expression of the gene in transgenic mice results in late prenatal lethality, suggesting that the dosage of its gene product is strictly controlled[4]. The *H19* gene maps to the distal segment of mouse chromosome 7, in a region that is parentally imprinted[5], a process by which genes are differentially expressed on the maternal and paternal chromosomes. We have now used an RNase protection assay that can distinguish between *H19* alleles in four subspecies of *Mus*, to demonstrate that the *H19* gene is parentally imprinted, with the active copy derived from the mother. This assay will be of general use in assaying allele-specific gene expression.

To determine whether a gene is imprinted, one must be able to distinguish expression from the maternal and paternal alleles. We used an RNase protection assay that is very sensitive to base substitutions and discontinuities in RNA sequences[6]. Hybrid F_1 mice generated from an interspecies cross between *Mus musculus domesticus* and *Mus spretus* were assayed for RNA levels transcribed from each *H19* allele. These two species diverged in evolution between 3 to 6 million years ago[7], and exhibit significant nucleotide polymorphisms, a property that has been especially useful in genetic mapping strategies[8]. For the *H19* gene, the fifth exon is especially divergent in sequence between humans and mice[3], and provides a likely candidate for harbouring an RNA polymorphism. An antisense RNA probe that included the 3′ end of exon 3, all of exon 4 and the 5′ part of exon 5 was derived from genomic DNA of the *M. domesticus* strain BALB/cJ (Fig. 1*a*). After hybridization to total liver RNA isolated from *M. domesticus* and *M. spretus* neonates, the RNase-resistant products were analysed by gel electrophoresis. *M. domesticus* RNA protected the expected full-length fragments corresponding to exons 3, 4 and 5. *M. spretus* RNA also fully protected exons 3 and 4 (date not shown), but a slightly

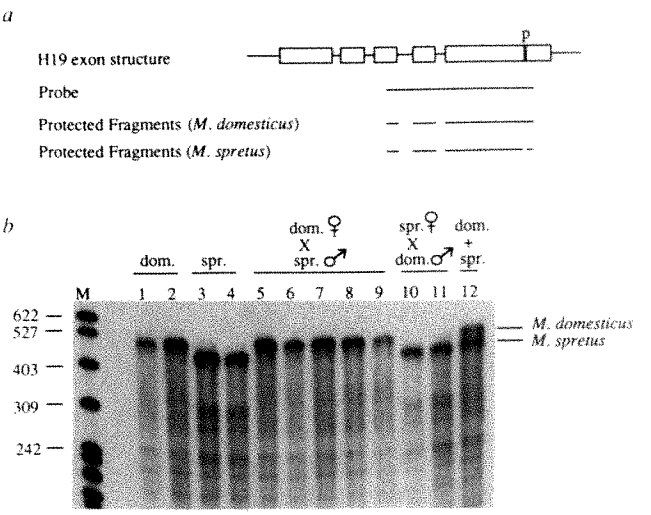


FIG. 1 RNase protection assay of liver RNA from *M. domesticus*, *M. spretus* and hybrid animals. *a*, The top line shows the exon structure of the mouse *H19* gene, drawn in a 5′ to 3′ orientation. Open boxes indicate exons and the lines correspond to introns and nontranscribed regions. The filled box in exon 5 and the corresponding 'p' above the box marks one possible site of the RNA polymorphism that exists between *M. domesticus* (dom.) and *M. spretus* (spr.). The second line shows a *Bam*HI–*Stu*I 754-base pair (bp) genomic DNA fragment that was used as the RNase protection probe. The probe fragments that are protected from RNase digestion following hybridization with *M. domesticus* or *M. spretus* H19 RNA are drawn in the third and fourth lines, respectively. *b*, Autoradiogram of RNase protection assay products of 2 μg total liver RNA samples from neonatal mice. The F_1 progeny of *M. domesticus* females mated to *M. spretus* males (lanes 5–9) and of *M. spretus* females mated to *M. domesticus* males (lanes 10 and 11), as well as the parental lines *M. domesticus* (lanes 1 and 2) and *M. spretus* (lanes 3 and 4), are as indicated. Only the allele-specific protected fragment from exon 5 is included. Marker lane (M) contains radioactively labelled DNA fragments of *Msp*I-digested pBR322, with relative sizes indicated in base pairs. Lane 12 shows an assay control using 1 μg *M. domesticus* RNA and 1 μg of *M. spretus* RNA. As both protected fragments display comparable intensities, the probe is equally capable of detecting both types of RNA. METHODS. The RNase protection was performed as described[6] with the following exceptions. The *Bam*HI–*Stu*I fragment of the *H19* gene was cloned into the *Bam*HI–*Hin*cII sites of pBluescript II KS (Stratagene). The vector was linearized with *Xba*I, purified by agarose gel electrophoresis and used as a template in an *in vitro* transcription reaction using [^{32}P]CTP and T3 polymerase (Promega). Total RNA was prepared from day 2–5 neonatal animals by the lithium chloride/urea method[19] and hybridized to 1×10^5 c.p.m. of radiolabelled probe overnight at 65°C. After RNase digestion at 32°C for 1 h, samples were analysed by electrophoresis on a 6.6% acrylamide, 7 M urea gel, and exposed to Kodak XAR-5 X-ray film.

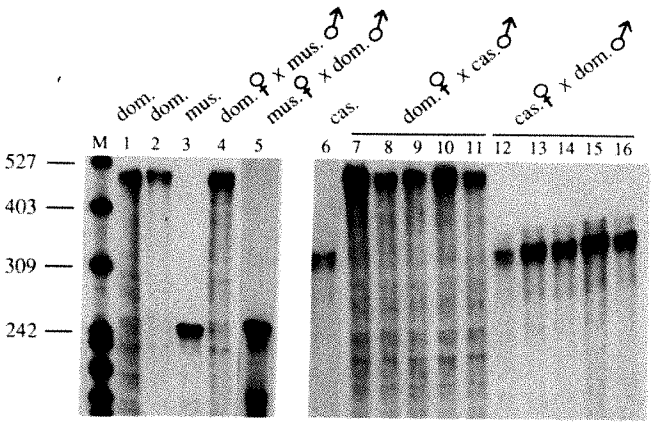


FIG. 2 RNase protection assay of skeletal muscle RNA from the progeny of *M. domesticus* crossed to either *M. castaneus* (cas.) or *M. musculus* (mus.). Total RNAs were isolated from skeletal muscle of 4-day-old *M. domesticus* (BTBR strain, 2 μg; lane 1), 3-month-old *M. domesticus* (CBA/NJX10/X10 strain, 10 μg; lane 2), 6-week-old *M. musculus* (SKIVE strain, 10 μg; lane 3), a 4-month-old F_1 mouse derived from a *M. domesticus* (CBA/NJX10/X10) female and *M. musculus* (Denmark) male cross (10 μg, lane 4) and a 6-week-old F_1 mouse from the reciprocal cross (10 μg, lane 5). Each RNA sample was subjected to the RNase protection assay as described in the legend to Fig. 1. Total RNA derived from a *M. castaneus* neonate (1 μg, lane 6), individual 3-week-old progeny of *M. domesticus* (C57BL/6J strain) females crossed to *M. castaneus* males (5 μg, lanes 7–11) and progeny of *M. castaneus* females mated to *M. domesticus* males (lanes 12–16) were treated similarly. The protected fragments correspond to exon 5.

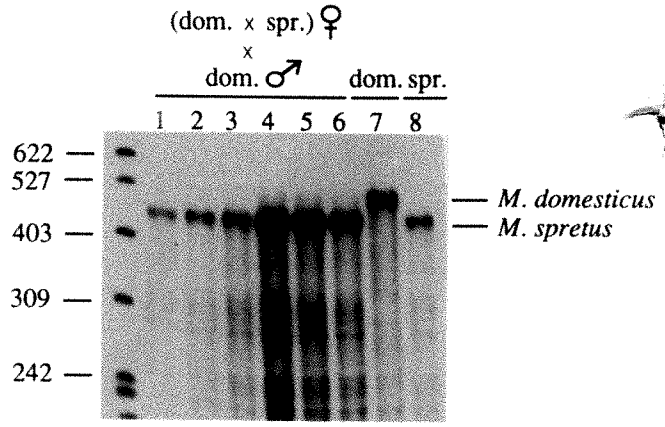


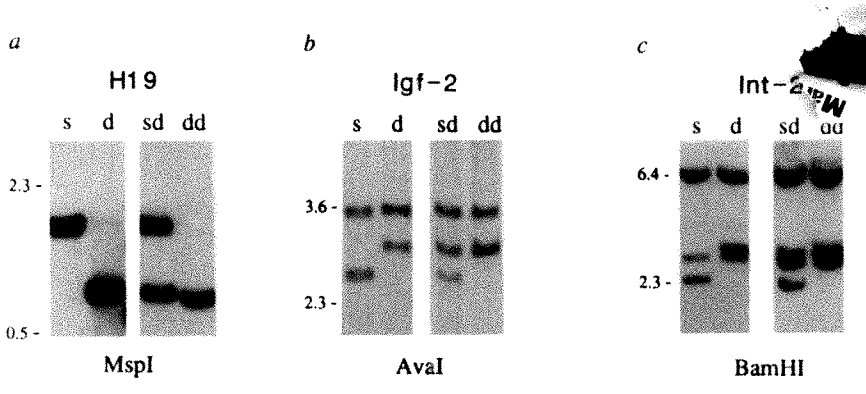
FIG. 3 Analysis of liver RNAs from *M. domesticus* × *M. spretus* backcross progeny. Female F_1 mice from a *M. domesticus* female × *M. spretus* male cross (Fig. 1) were mated with *M. domesticus* males and progeny were killed at different ages after birth. Total liver RNA isolated from progeny identified as being heterozygous for *H19* were assayed by RNase protection as described in Fig. 1: 1 day (1 μg, lanes 1 and 2), 4 days (2 μg, lane 3) and 1 week (5 μg, lanes 4–6). Liver RNA from homozygous *M. domesticus* and *M. spretus* was also assayed (2 μg, each, lanes 7 and 8, respectively). The protected fragments correspond to exon 5.

smaller exon 5 fragment was observed (Fig. 1*b*, compare lanes 1 and 2 to lanes 3 and 4).

When liver RNAs from neonatal progeny of *M. domesticus* females mated with *M. spretus* males were tested with the RNase protection assay, only the *H19* gene product characteristic of *M. domesticus* exon 5 was observed (Fig. 1*b*, lanes 5–9). Conversely, the reciprocal cross using *M. domesticus* males and *M. spretus* females resulted in progeny that expressed only the *M. spretus H19* allele (Fig. 1*b*, lanes 10 and 11). In both cases, only the allele inherited from the mother was expressed.

The exclusive expression of the *H19* maternal allele could be a unique property of the mouse strains used in this interspecific cross. We therefore analysed two additional crosses using different *Mus* species. Skeletal muscle RNAs from 2–3-month-old progeny from *M. domesticus* × *Mus musculus musculus* reciprocal crosses were assayed for the presence of H19 RNA. The *M. musculus* RNA protects a fragment of about 240 bases, easily distinguished from the *M. domesticus* product (Fig. 2, lanes 1–3). Analysis of the hybrid progeny confirmed that only the maternally derived allele was expressed (Fig. 2, lanes 4 and 5).

Likewise, when skeletal muscle RNAs derived from F_1 progeny of *M. domesticus* × *Mus musculus castaneus* reciprocal crosses were analysed, the maternal allele was once again exclusively detected (Fig. 2, lanes 7–16). In addition to verifying the parental imprinting of the *H19* gene, these experiments illustrate that cells of both liver and skeletal muscle, derived from two



Recombination interval	Number of recombinants	Map distance (cM)
H19 - Int-2	0/110	<2.7
Igf-2 - Int-2	0/109	<2.7

FIG. 4 Genetic mapping of the *H19* gene. *a*, *b* and *c*, Southern blots identifying RFLPs between *M. spretus* and *M. domesticus* using a 1.1-kb *Bam*H1 *H19* genomic DNA fragment[1], a 550-bp rat *Igf-2* cDNA[20], and a 1.0-kb mouse *Int-2* cDNA[21], respectively. In each panel the first two lanes show *M. spretus* (s) and *M. domesticus* (d; BALB/cJ strain) parental DNAs and the last two lanes show heterozygous (sd) and homozygous (dd) DNA from a (s × d) × d backcross. The restriction endonuclease used to digest the DNA is designated under each panel and relative size standards (in kb) are shown to the left. *d*, Number of recombinants detected between the *Int-2* and *H19* or *Igf-2* genes. The genetic distance represents the maximum distance defined at the 95% confidence level, calculated using the formula $0.05 = (1-p)^N$, where p is map distance in Morgans and N is number of animals tested.

METHODS. Genomic DNA (15 μg) was digested and separated on a 1.0% agarose gel. DNA was transferred to Genescreen (NEN) by capillary transfer in 0.2 N NaOH/0.6 N NaCl. The filters were neutralized in 1 M Tris–HCl (pH 7.5), hybridized overnight using the conditions described by Church and Gilbert[22] and washed for 2 h at 65°C in 0.015 M NaCl–0.0015 M sodium citrate/0.1% SDS. The probes were prepared by the method of Feinberg and Vogelstein[23] using [^{32}P]dCTP.

different germ layers, display similar imprinting patterns, as does the neonatal gut (data not shown).

If the *H19* gene is indeed parentally imprinted, the pattern of allele-specific expression will be reset in the germ line at each generation. To test this, female F_1 (*M. domesticus* × *M. spretus*) mice, expressing the *M. domesticus H19* allele (Fig. 1*b*, lanes 5–9), were backcrossed to *M. domesticus* males. The progeny that inherited the *M. spretus H19* allele from the mother were identified by restriction fragment length polymorphism analysis of genomic DNA (data not shown). Analysis by RNase protection of liver RNAs from the progeny demonstrated that the maternally derived *M. spretus* allele was exclusively expressed (Fig. 3, lanes 1–6). This result demonstrates that the formerly silent *M. spretus* allele inherited from the mother was reactivated during oogenesis. A total of 24 backcross progeny were screened, and all displayed the exclusive expression of the *M. spretus* maternal allele. From this we also conclude that the imprinting requires sequences that are linked to the *H19* gene itself. In addition, the segregation of unlinked modifiers of imprinting, which can occur for imprinted transgenes[9,10], was not detected.

Both the *H19* gene[1] and the insulin-like growth factor-II gene (*Igf-2*) (T. Glaser and D. Housman, personal communication) map to mouse chromosome 7. Recently DeChiara *et al.* deleted the *Igf-2* gene by homologous recombination in embryonic stem cells[11]. Heterozygotes who had inherited this inactive *Igf-2* gene from the father were smaller than normal. When the inactive gene was inherited from the mother, however, the heterozygote progeny were of normal size[12]. The authors conclude that only the paternal allele of *Igf-2* is active in most expressing tissues, with the exception of the choroid plexus and the leptomeninges, where both alleles are active.

We used an interspecific backcross between the *M. domesticus* BALB/cJ strain and *M. spretus* to determine the genetic linkage between *H19* and *Igf-2*. Restriction fragment length polymorphisms that distinguish the alleles of the two genes (Fig. 4*a–c*) were assayed in the genomic DNAs of 110 progeny of a (BALB/cJ × *M. spretus*) × BALB/cJ backcross. No recombinants were observed (Fig. 4*d*). To position the genes on the chromosome 7 map, they were mapped relative to *Int-2*, which encodes a member of the fibroblast growth factor gene family, and which has been mapped to the distal third of chromosome 7 (ref. 13). No recombinants between *Int-2*, *H19* and *Igf-2* were detected, indicating that these genes are tightly linked. *H19* and *Igf-2* have been mapped to band 11p15.5 in the human genome[14]. This syntenic conservation would tend to discount our failure to detect recombinants being the consequence of an inversion on distal chromosome 7 of *M. spretus* relative to *M. domesticus*[15].

That the *H19* and *Igf-2* genes are tightly linked, yet imprinted in opposite directions, implies that the signals for parental imprinting are unlikely to act over large chromosomal regions, in contrast to X chromosome inactivation[16]. Indeed, Barlow *et al.* showed that the *Igf-2r* gene, encoding the receptor for insulin-like growth factor-II, is expressed only from the maternally inherited chromosome 17 (ref. 17). But three other genes within a region of about 1.1 megabases containing *Igf-2r* are not subject to imprinting.

That the *H19* gene undergoes parental imprinting was suggested by two lines of evidence. First, introduction of additional copies of the *H19* gene in transgenic mice leads to late prenatal lethality, between 14 and 16 days of gestation[4]. Second, *H19* maps to a region which is parentally imprinted[5]. Using balanced translocations, Searle and Beechey demonstrated that a maternal duplication/paternal deficiency of distal chromosome 7 results in late prenatal lethality, whereas paternal duplication/maternal deficiency leads to early embryonic lethality[18]. The remarkable similarity in the timing of the lethal effects of extra copies of the *H19* gene in the transgenic experiments and the maternal duplications of chromosome 7 suggest strongly that they share the same aetiology: one or more extra copies of an active *H19* gene. Whether this is the true cause of the lethality remains to be determined. The absence of *Igf-2* gene function, which would be the case in mice carrying the maternal duplications/paternal deficiencies, results in smaller mice, and therefore it alone cannot explain the prenatal lethality[12].

Whether the early embryonic lethality observed with paternal duplications/maternal deficiencies can be explained by loss of *H19* gene function will require the generation of such mutations through gene replacement in embryonic stem cells. Nevertheless, the studies described here are consistent with an important role for the *H19* gene in embryonic development in the mouse. That role remains unclear, however, given the unusual nature of the gene product, which is found in a highly abundant cytoplasmic RNA complex[3]. That even one extra copy of the gene may have specific deleterious effects implies that its abundance is being carefully controlled. □

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Uptake of *Pneumocystis carinii* mediated by the macrophage mannose receptor

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HUMAN exposure to *Pneumocystis carinii* is common[1,2] but, in the absence of acquired[3] or genetic[4] dysfunction of either cellular or humoral immunity, exposure rarely leads to illness. Although alveolar macrophages can degrade *P. carinii*[5,6], macrophage receptors involved in *P. carinii* recognition have not been clearly defined.

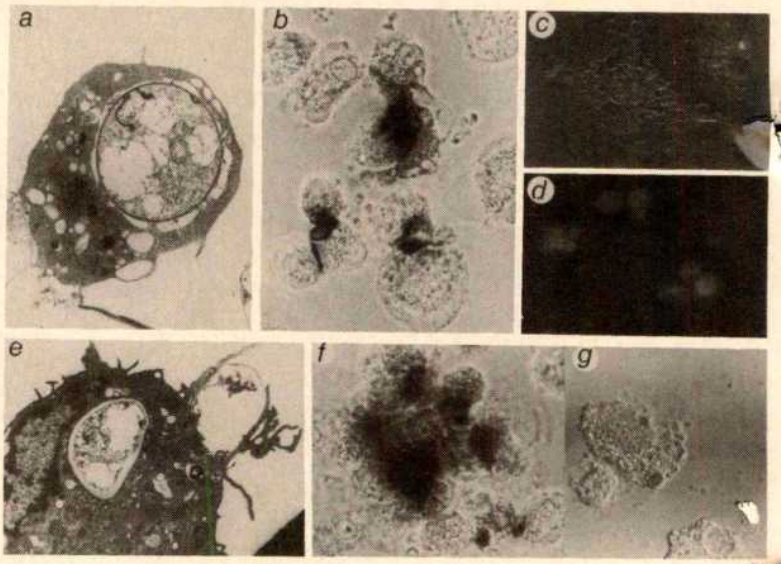

FIG. 1 *a*, An electron micrograph of rat alveolar macrophage that has ingested a typical thick-walled cyst form of *P. carinii*. *b*, *f*, Reduction of nitroblue tetrazolium, a histochemical dye that measures superoxide release, is triggered around labelled (*b*) and unlabelled (*f*) *P. carinii* after incubation with human alveolar macrophages. Organisms in a ratio of 10 organisms to one macrophage were incubated for 30 min at 37 °C. In parallel experiments, quantitative superoxide release was measured by the cytochrome *c* reduction assay[13] and this resulted in 6 nmol O_2 release by 8×10^5 cells per 60 min. *c*, Human alveolar macrophages bind and ingest *P. carinii* as shown by differential interference contrast microscopy. *d*, The same field as in *c* viewed by fluorescence microscopy demonstrates that the organisms are readily visible, greatly enhancing accurate single-cell quantitation. *e*, Electron micrograph of rat alveolar macrophage having ingested an unlabelled *P. carinii*. *g*, Human alveolar bind and ingest unlabelled *P. carinii* as viewed by difference interference microscopy, but the unlabelled organisms are difficult to visualize.
METHODS. *P. carinii* were derived from the lungs of male Sprague–Dawley rats (Hilltop Laboratory Animals, Pennsylvania). After isolation they were propagated and purified in short-term cell culture as previously described[10,21]. *P. carinii* from cell culture supernatants were washed three times in PBS, resuspended to 1×10^7 per ml in PBS containing 0.1 mg ml^{-1} FITC (Sigma) and incubated at 37 °C for 30 min. They were then washed three times in PBS (10 ml at 4° or 22 °C for 20 min, then spun down at 900*g*) and stored in PBS at 4 °C in the dark until use. To avoid clumping, the organisms were not resuspended in complex media until just before use.

Characterization of a predominant surface glycoprotein of the high mannose type[7,8] led us to investigate the role of the macrophage mannose receptor in this process. We report here that binding and uptake of cultured rat *P. carinii* by human and rat alveolar macrophages is reduced by 90% in the presence of competitive inhibitors of mannose receptor activity and by adherence of alveolar macrophages to mannan-coated surfaces. Further, only those COS cells transfected with the human macrophage mannose receptor complementary DNA that express surface mannose receptors bind and ingest *P. carinii*. These studies establish that the macrophage mannose receptor is sufficient for uptake of *P. carinii* and emphasize the role of the alveolar macrophage in first-line host defence against *P. carinii*.

Because of the small size and refractility of unstained *P. carinii* organisms, they are difficult to see by light microscopy[9]. We thus devised an assay using fluoresceinated *P. carinii*. As fluorescein may alter the net surface charge on the organisms, we first had to validate that these labelled organisms are an effective substrate for phagocytosis. Rat and human alveolar macrophages expressing 5×10^5 per cell (ref. 10) were isolated by bronchoalveolar lavage and plated on glass coverslips. Cell monolayers were then incubated with *P. carinii* which had been propagated in short-term culture[9]. Phagocytosis of unlabelled *P. carinii* and organisms that had been labelled using the method described in the legend to Fig. 1 were compared. Figure 1*a*, *c*, and *d* shows that labelled organisms were taken up by alveolar macrophages and are indistinguishable from the unlabelled organisms (Fig. 1*e* and *g*). Labelling the organisms helped single-cell quantitation by allowing both fluorescence microscopy and differential interference contrast microscopy (Fig. 1*c* and *d*) to be used to distinguish bound versus ingested particles. The labelled organisms, like untreated organisms, bound to macrophages and some were also ingested (Fig. 1*a*, *c*, *d*, labelled; Fig. 1*e*, *g*, unlabelled). Studies with human alveolar macrophages in which 200 cells on three independent coverslips incubated with a ratio of 10:1 unlabelled or labelled organisms for 1 h gave a phagocytic index of 510 ± 60 and 540 ± 40, respectively, with an average of five organisms per cell.

We next compared the ability of labelled and unlabelled *P. carinii* to trigger macrophage release of reactive oxygen intermediates. Labelled (Fig. 1*b*) and unlabelled *P. carinii* (Fig. 1*f*) triggered the release of superoxides, as shown by the dye nitroblue tetrazolium, which formed insoluble blue deposits around the organisms. Both labelled and unlabelled organisms triggered the release of comparable amounts of superoxide after 1 h incubation at 37 °C (6 nmol and 6.5 nmol, respectively). Labelling did not seem to alter the ability of the organisms to serve as substrates for phagocytosis or to trigger a respiratory burst, and therefore provides a simple and qualitative method for studying the binding of *P. carinii* to the macrophage mannose receptor.

Rat alveolar macrophages were cultivated for 1 h at 37 °C in the presence of several concentrations of soluble competitive inhibitors of the mannose receptor. Dose-dependent inhibition

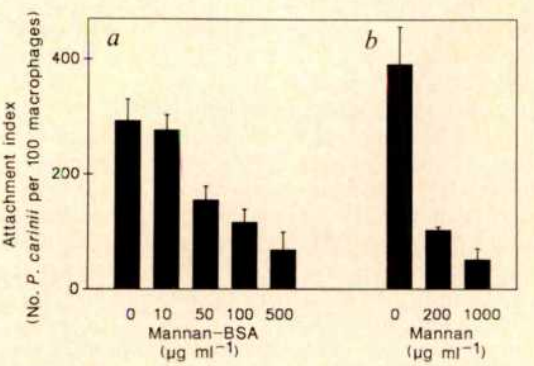

FIG. 2 Inhibition of binding of fluoresceinated *P. carinii* to rat alveolar macrophages by mannosylated BSA (*a*) and yeast mannan (*b*).
METHODS. Rat alveolar macrophages were isolated by standard lavage and plated on Lab-Tek 8-well slide chambers at 5×10^4 cells per well in RPMI plus 10% FCS. The cells were cultured for 24 h at 37 °C in 5% CO_2, washed in RPMI without serum and the indicated concentration of mannosylated BSA (E-Y Labs) or yeast mannan (Sigma) added for 20 min. Fluoresceinated *P. carinii* were then added at a final ratio of 20:1 (*P. carinii* to macrophages) and incubated for a further 60 min (final volume 0.2 ml) at 37 °C. The slides were then washed five times in PBS, fixed in 2% paraformaldehyde and the number of *P. carinii* associated per 100 macrophages counted in triplicate chambers by phase and fluorescent microscopy.

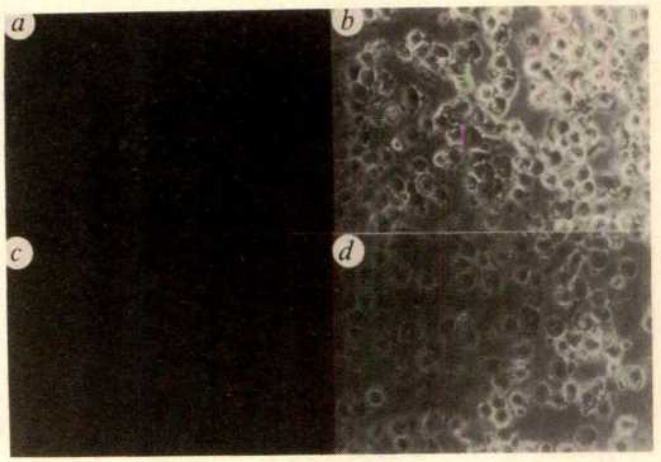

FIG. 3 Modulation of macrophage mannose receptor by mannan-coated coverslips, but not those coated with bovine serum albumin, reduced binding and uptake of fluoresceinated *P. carinii* by 90%. Substrata were prepared as described[12,13]. Macrophages adhered to mannan surfaces showed 80% reduction in binding of ^{125}I–mannose–BSA. *a*, Fifty per cent of 2×10^5 human alveolar macrophages adhering to BSA-coated coverslips bound or ingested one or more fluoresceinated *P. carinii* (target to cell ratio 10:1; incubation time 40 min at 37 °C). *b*, Phase contrast micrograph of the same field. *c*, When they adhered to a mannan-coated substrate, less than 5% of human alveolar macrophages had bound or ingested one *P. carinii*. *d*, Phase contrast micrograph of the same field reveals a healthy cell monolayer. Cells adherent to mannan coverslips had 80% reduction in apical surface mannose receptors as detected by reduced binding of ^{125}I–mannose–BSA (not shown) and were able to ingest opsonized *Candida albicans*, indicating that the decreased binding and ingestion was selective. The methods used have been described elsewhere[12,13].

of *P. carinii* cell attachment was observed with both mannose–BSA, a neoglycoprotein (37 mol mannose: 1 mol BSA)[11], and mannose-rich yeast wall mannan extract (Fig. 2). There was 85% inhibition with 500 μg ml^{-1} of human BSA and 90% inhibition with 1 mg ml^{-1} of mannan. In each experiment at least 300 cells were scored on triplicate slides. Mannose receptors were then selectively depleted from the macrophage plasma membrane by adherence to mannan-coated coverslips[12,13]. Human alveolar macrophages adhering to mannan-coated coverslips had a 90% reduction in cell-associated *P. carinii* compared with macrophages plated on BSA substrata (Fig. 3*c* and *a*, respectively). Although macrophages adherent to mannan substrates had greatly reduced numbers of bound and ingested labelled organisms, the cells had a typical macrophage appearance (Fig. 3*b* and *d*) and could ingest fluorescein isothiocyanate latex beads (not shown).

The recognition of other microorganisms by macrophages often involves cooperation between a number of cell-surface receptors on the macrophage surface[14]. A role for fibronectin in *P. carinii* cell adherence to epithelial cells and macrophages has been shown[15,16]. To confirm that the mannose receptor was sufficient for *P. carinii* uptake, we examined COS-I cells that had been transfected with a full-length mannose receptor cDNA[17,18]. This showed that a subpopulation of COS cells

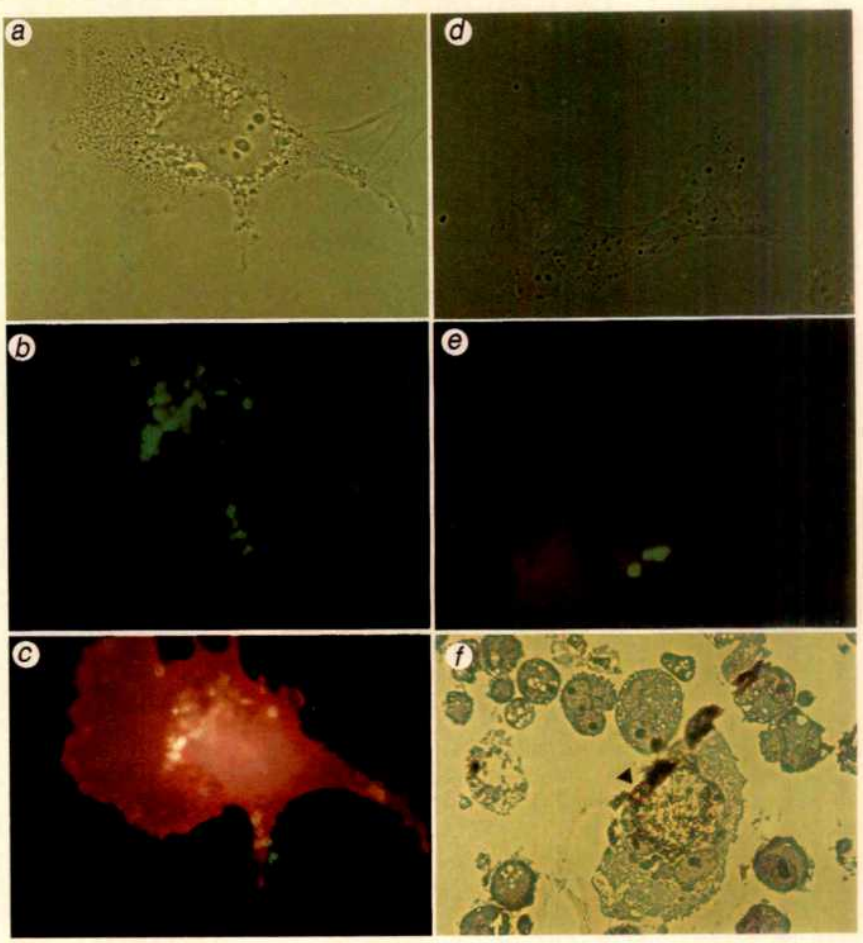

FIG. 4 Mannose receptor transfected COS-I cells are able to bind and ingest *P. carinii*. *a*, Phase contrast micrograph of COS-I cell that had been transfected with a full-length macrophage mannose receptor cDNA[17,22] and incubated with fluoresceinated *P. carinii* (cell to target ratio 15:1) for 30 min at 37 °C. The coverslips were then fixed with 3.5% formaldehyde and stained with a 1:100 dilution of a rabbit anti-mannose receptor antisera detected by an affinity-purified goat anti-rabbit rhodamine coupled immunoglobulin (Tago, California, USA). Mock transfected and untransfected COS cells failed to bind *P. carinii*. *b*, Photomicrograph of the same field as viewed under fluorescence microscopy. The *P. carinii* are more easily seen and appear green. *c*, Localization of mannose receptors and *P. carinii* as determined by the yellow staining of the majority of organisms. This results from the mixture of fluorescein and rhodamine. One organism remains green and is clearly extracellular. The cell shown is representative of all cells that had bound or ingested *P. carinii*. Of note is that only those cells that stained for the mannose receptor had associated with bound organisms. This constituted 20–30% of transfectants, which is the percentage of cells expressing mannose receptors as determined by FACS analysis. *d*, Phase contrast micrograph of mannose receptor-transfected COS-I cell that had been incubated with fluoresceinated *P. carinii* as described above, fixed and stained with normal rabbit antisera followed by a goat anti-rabbit rhodamine coupled immunoglobulin as a control for the specificity of the rhodamine coupled antibody. *e*, Dual fluorescence range of the field shown in *d* reveals green fluoresceinated organisms and a low background red stain. Exposure times were identical to those for *c*. *f*, Light micrograph of a 500-nm section showing unlabelled cysts within a COS cell transfected with mannose receptor cDNA. Sections were stained with toluidine blue which stains cysts and cell nuclei. Arrow indicates ingested *P. carinii*.

transfected with mannose receptor cDNA had specifically bound and ingested *P. carinii* (Fig. 4*a*, *b*). Double-fluorescence experiments revealed only those cells expressing surface mannose receptor had associated organisms. Colocalization of labelled organisms with mannose receptor (detected by rhodamine) membrane segments produced a yellow image (Fig. 4*c*). One organism still appeared green and is clearly not cell-associated. Another organism appeared half green (outside) and the other half is double-stained suggesting that the mannose receptor-containing membrane associated with the organism was in the process of enveloping the bound parasite (Fig. 4*c*). Controls for the specificity of the rhodamine staining (Fig. 4*d*) show phase contrast and double fluorescence of cells incubated with nonimmune rabbit sera followed by rhodamine-coupled second antibody (Fig. 4*e*). One aspect of the COS cells expressing the mannose receptor is that they were able to ingest the attached *P. carinii* (Fig. 4*f*). The ability of the mannose receptor to mediate phagocytosis in heterologous cells is consistent with previous experiments 17.

In the immunocompetent host, the small numbers of organisms inhaled may be engaged by alveolar macrophage mannose receptors which result in release of reactive oxygen-intermediates and engulfment of the bound particle. But in immunocompromised states in man or experimental animal[19,20], an environment is created that favours the proliferation of *P. carinii* and the establishment of an extracellular infectious focus. Many factors may contribute to the failure of alveolar macrophages to clear the infection under these circumstances. It is possible that the mannose-rich surface proteins shed in *P. carinii* infection downregulate the expression and activity of mannose receptor, as has been shown with the mannose-rich yeast particle zymosan[18]. These abundant cell-surface proteins with high-mannose glycans may contribute to the pathobiology of *P. carinii* and may provide targets for therapeutic preparations of soluble forms of the mannose receptor or mannose-binding proteins. □

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Dependence of Ypt1 and Sec4 membrane attachment on Bet2

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MANY small GTP-binding proteins are synthesized as soluble proteins that are post-translationally modified as a prerequisite for membrane attachment[1]. Ypt1 and Sec4 are homologous Ras-like GTP-binding proteins that have been proposed to regulate the specificity of vesicular traffic at different stages of the secretory pathway by cycling on and off membranes[2–6]. Here we show that *BET2*, initially identified as a gene required for transport from endoplasmic reticulum to Golgi apparatus in yeast[7], encodes a factor that is needed for the membrane attachment of Ypt1 and Sec4. DNA sequence analysis has revealed that Bet2 is homologous to Dpr1 (Ram1), an essential component of a protein prenyltransferase that modifies Ras[8], enabling it to attach to membranes[9,10]. We propose that Bet2 modifies Ypt1 and Sec4 in an analogous manner.

We determined whether the *BET2* gene interacts with other genes whose products mediate secretion in yeast by crossing *bet2* to a representative of each of the complementation groups of secretory mutants that block vesicular transport from the endoplasmic reticulum to the Golgi complex (*bet1*, *sec12*, *sec13*, *sec16*, *sec17*, *sec18*, *sec20*, *sec21*, *sec22*, *sec23*), through the Golgi apparatus (*sec7*, *sec14*), and from the Golgi to the cell surface (*sec1*, *sec2*, *sec3*, *sec4*, *sec5*, *sec6*, *sec8*, *sec9*, *sec10*, *sec15*). We found that double mutant strains containing *bet2-1* in combination with *sec4-8* or with a mutation in genes (*sec2-41*, *sec5-24*, *sec8-9* and *sec15-1*) that strongly interact with *sec4-8* (ref. 5), are inviable or nearly inviable at a temperature at which each single mutant grows well (25 °C, see Table 1). The same phenomenon was also observed when *bet2-1* was combined with *ypt1-2* (ref. 3), or *sec21-1*, a mutation in a gene that interacts genetically with *ypt1-2* (ref. 3). Therefore, in addition to interacting genetically with *YPT1*, *BET2* interacts with *SEC4* and with those genes previously shown to interact with either *YPT1* or *SEC4*. These findings could reflect a physical interaction between Bet2 and both Ypt1 and Sec4. Alternatively, Bet2 may perform a similar function on a pathway parallel to that of Ypt1 and Sec4.

One explanation for these genetic findings is that Bet2 is necessary to maintain the function of both Ypt1 and Sec4. As many GTP-binding proteins function when membrane-bound, they may be defective for function in the *bet2-1* mutant because they fail to attach to membranes. To test this hypothesis, the membrane association of Ypt1 and Sec4 was examined in wild-type and mutant cells after incubation at either 25 °C or 37 °C. At either temperature, the soluble pool of both proteins (Fig. 1*a*, lanes 5 and 2, *b*, lanes 7 and 3) was substantially larger in the mutant than in the wild type, and the fraction associated with membranes (Fig. 1*a*, lanes 6 and 3, *b*, lanes 8 and 4) was nearly absent or largely diminished (see legend to Fig. 1). For Ypt1, we also observed the presence of a degradation product in the soluble pool of wild type (Fig. 1*a*, lanes 1 and 2) which was reduced in quantity in the mutant (Fig. 1*a*, lanes 4 and 5). The failure of Ypt1 to attach to membranes was not an indirect consequence of blocking traffic from endoplasmic reticulum to Golgi or traffic within the Golgi apparatus, as its association with membranes was still seen in other secretory mutants that block transit at this stage of the pathway (not shown). These localization studies predict that, in the *bet2-1* mutant, loss of function will be more severe for Ypt1 than Sec4. The phenotypic analysis of this mutant, described below, shows this to be so. The Bet2 protein does not mediate the membrane attachment of all small GTP-binding proteins. Rather, it seems to function in conjunction with a particular class of proteins, as the localiz-

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ation of Ras2 (Fig. 1*b*, compare lanes 1, 3 and 4 with lanes 5, 7 and 8), as well as Ras1 (not shown), is unaltered in this mutant.

To demonstrate that the activity of Bet2 causes Ypt1 to attach to membranes, we examined the distribution of newly synthesized protein in a strain carrying a second mutant allele of this gene, *bet2-2* (previously identified as *orf2-1*)[11]. Although we found that Ypt1 was attached to membranes in permissively grown *bet2-2* cells (Fig. 1*c*, compare lanes 4, 5 and 6 with 1, 2 and 3), it was completely soluble if the cells were incubated at 37 °C before fractionation (Fig. 1*c*, lanes 7, 8 and 9). This indicates that, in the *bet2-2* mutant, the attachment of *Ypt1* to membranes is temperature-sensitive. In contrast, the membrane association of Ypt1 is defective at both 25 °C and 37 °C in the *bet2-1* strain. At 25 °C, the growth of *bet2-1* is not severely impaired. Death does, however, ensue when the cells are shifted to 37 °C. One explanation for this phenomenon is that the requirement for Ypt1 and Sec4 function is increased at 37 °C, exacerbating the constitutive defect of *bet2-1* at this raised temperature.

If, as we propose, the Ypt1 protein is defective for function in the *bet2* mutant, then this mutant should display the properties of a *ypt1* mutant. As for *ypt1-2* (ref. 3), *bet2-1* mutant fractions fail to support the late events that take place in an *in vitro* assay (not shown) that reconstitutes endoplasmic reticulum to Golgi and intra-Golgi transport[12,13]. In addition, we compared the form of invertase secreted at 37 °C by *bet2-1* with that secreted by *ypt1-1* mutant cells (Fig. 2). In the *ypt1-1* mutant, the core glycosylated or endoplasmic reticulum form of invertase accumulates intracellularly at the restrictive temperature, whereas an underglycosylated form of this protein is secreted[3] (also see Fig. 2, lanes 6 and 5, compare lanes 2 and 1). The *bet2-1* mutant also has this phenotype (Fig. 2, lanes 4 and 3). Thus, the invertase that fails to accumulate in the endoplasmic reticulum lumen of *bet2-1*, does not collect in post-Golgi secretory vesicles as it does in *sec4*, but instead is secreted as an underglycosylated protein as in the *ypt1-1* mutant.

One aspect in which Ypt1 and Sec4 differ from yeast Ras is that they have a CC motif at their carboxy terminus rather than

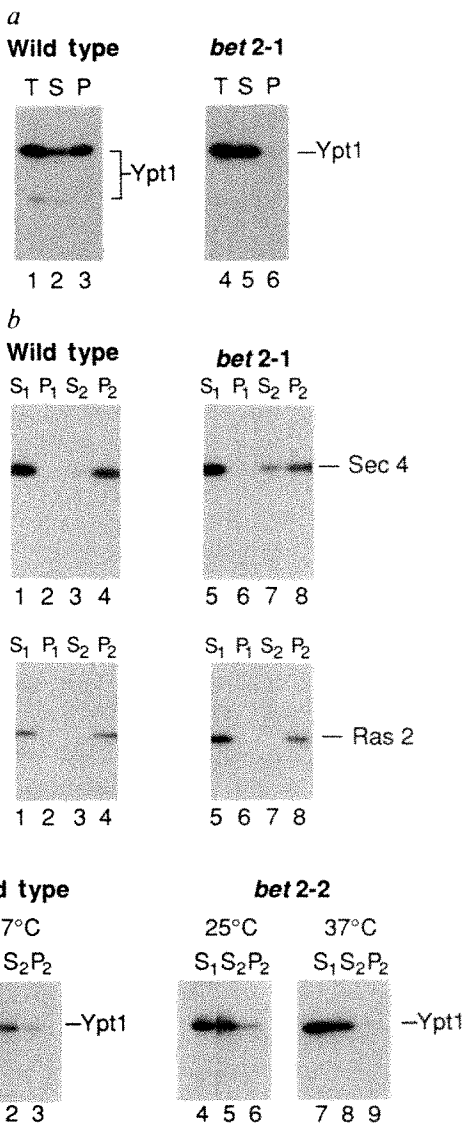

FIG. 1 A defect in the Bet2 protein leads to an increase in the soluble pool of Ypt1 and Sec4 but not Ras. *a*, Lane 1, wild-type lysate (T); lane 2, wild-type soluble fraction (S); lane 3, wild-type insoluble fraction (P); lane 4, *bet2-1* mutant lysate (T); lane 5, *bet2-1* soluble fraction (S); lane 6, *bet2-1* insoluble fraction (P). *b*, Lane 1, supernatant of wild-type lysate subsequent to a spin at 450*g* (S_1); lane 2, pellet from the 450*g* spin (P_1); lane 3, wild-type soluble fraction (S_2); lane 4, wild-type insoluble fraction (P_2); lane 5, S_1 from *bet2-1*; lane 6, P_1 from *bet2-1*; lane 7, S_2 from *bet2-1*; lane 8, P_2 from *bet2-1*. *c*, Lane 1, S_1 from wild type (37 °C); lane 2, S_2 from wild type (37 °C); lane 3, P_2 from wild type (37 °C); lane 4, S_1 from *bet2-2* (25 °C); lane 5, S_2 from *bet2-2* (25 °C); lane 6, P_2 from *bet2-2* (25 °C); lane 7, S_1 from *bet2-2* (37 °C); lane 8, S_2 from *bet2-2* (37 °C); lane 9, P_2 from *bet2-2* (37 °C).
METHODS. *a*, Wild-type (SFNY26-6A; *MATα, his4-619*) and *bet2-1* (ANY119; *MATα, bet2-1, ura3-52, his4-619*) mutant cells were grown overnight at 25 °C in yeast peptone dextrose medium to an early exponential phase. One sample of cells ($150A_{599}$ units) was converted to spheroplasts during incubation for $1\frac{1}{2}$ h at 25 °C in spheroplast medium[7]. A second was incubated at 37 °C for 1 h and then converted to spheroplasts during 1 h incubation at 37 °C. The spheroplasts were washed, lysed in 6 ml ice cold lysis buffer described before[18], and the lysate (T) was homogenized with a Wheaton tissue grinder. Unbroken cells were removed during a 3min spin at 450*g* and the supernatant of this spin (S_1) was centrifuged at 200,000*g* for 1 h to generate a soluble fraction (S or S_2). The pellet (P or P_2) was resuspended in a volume of lysis buffer equal to the supernatant. Samples were electrophoresed and western blotted using anit-Ypt1 antibody. A degradation product of the Ypt1 protein (M_r 21,000), is seen on the blot shown in *a*. The Ypt1 protein has an M_r of 23,000. The blots from several experiments were measured and the percentages of the total (and standard deviations) are given in this legend. From these blots we determined that the soluble pool in the mutant (84.3 ± 1.15%) was substantially larger when compared to wild type (46.3 ± 6.8%)[15], whereas the insoluble fraction was nearly absent (7.66 ± 2.5% in the mutant compared to 36.66 ± 11% for wild type). The results obtained were independent of the temperature of the incubation. *b*, Wild-type (SFNY 96; *MATa, ura3-52,* pYCp50-*ADH RAS2*) *and bet2-1* mutant cells (SFNY 98; *MATα, bet2-1, ura3-52, his4-619,* YCp50-*ADH RAS2*), harbouring the *RAS2* gene on a YCp50-ADH vector, were fractionated as described above. Samples were electrophoresed and western blotted using either anti-Sec4, or anti-Ras antibody, as described before[18,22]. When the blots from several experiments were measured, the soluble pool of Sec4 in the mutant was found to be larger (48.4 ± 8.57%) when compared to wild type (17.2 ± 7.9%)[18], whereas the insoluble fraction was greatly reduced (38.6 ± 8.23% compared to 79.2 ± 3.56% for wild type). The results obtained for the Sec4 protein were independent of the temperature at which the incubation was performed. *c*, To analyse the distribution of newly synthesized Ypt1 protein, wild-type (YF1593)[11] and *bet2-2* mutant cells (YF1594)[11] were grown overnight at 25 °C and radiolabelled at the permissive and restrictive temperatures. A portion of each culture ($4A_{599}$ units) was labelled with 250 μCi of trans [^{35}S]label at 25 °C (10 min); a second aliquot of cells ($4A_{599}$ units) was radiolabelled for 10 min at 37 °C, after incubation (15 min or 1 h) at this temperature. (The same results were obtained with preincubation at 37 °C for either 15 min or 1 h.) All samples were then fractionated, as described above, and Ypt1 was immunoprecipitated from each fraction[7]. The distribution of Ypt1 in wild type was identical when the cells were incubated at either 25 °C (not shown) or 37 °C (lanes 1, 2 and 3). The wild-type soluble pool was, however, found to be larger (~2/3 is soluble and 1/3 is membrane bound) than reported in the legend to *a*. This variation may be a consequence of the strain background, because the wild-type strains used in the experiments shown in *a* and *c* were nonisogenic. The data in *c* is representative, as this experiment was repeated several times and the results were always identical. We have also examined the membrane association of newly synthesized Ypt1 in the *bet2-1* mutant (ANY 119) and SFNY 26-6A at 25 °C and 37 °C and found the distribution of this protein to be the same as reported in *a*.

TABLE 1 Tetrad analysis

	Number of tetrads							
	Four*			Three†			Two‡	
	4−:0+§	3−:1+	2−:2+	3−:0+	2−:1+	1−:2+	2−:0+	1−:1+	0−:2+
bet2-1 crossed with									
sec2-41	6				11		1	2	5
sec4-8	4				15	1			3
sec5-24	3				7			2	1
sec8-9	4	1			14	1			2
sec15-1	5				5				2
sec21-1					8				3

When *bet2* was crossed to the yeast secretory mutants listed above, either four, three or two of the four spores obtained were unimpaired for growth at 25 °C. The spores impaired for growth always contained both secretory mutations in the same haploid strain. This was revealed by analysis of the remaining spores in each tetrad. Haploid double mutant strains containing *bet2-1* in combination with either *sec4-8, sec5-24, sec8-9, sec15-1 or sec21-1* were nearly invariable at 25 °C. Double mutants containing *bet2-1* in combination with *sec2-41* were inviable. Strains containing *bet2-1* in combination with either *sec1-1, sec6-4, sec7-1, sec12-4, sec*13-1, *sec14-3, sec16-2, sec17-1, sec18-1, sec22-3, sec23-1* or *bet1-1* were not impaired for growth; small growth defects were observed at 25 °C when *bet2-1* was combined with either *sec3-2, sec9-4, sec10-2, sec19-1* or *sec20-1* in the same haploid strain.

* All four spores exhibited unimpaired growth at 25 °C.

† Three of the four spores exhibited unimpaired growth at 25 °C.

‡ Two of the four spores exhibited unimpaired growth at 25 °C.

§ Designates the number of spores that grew (+) or did not grow (−) at 37 °C.

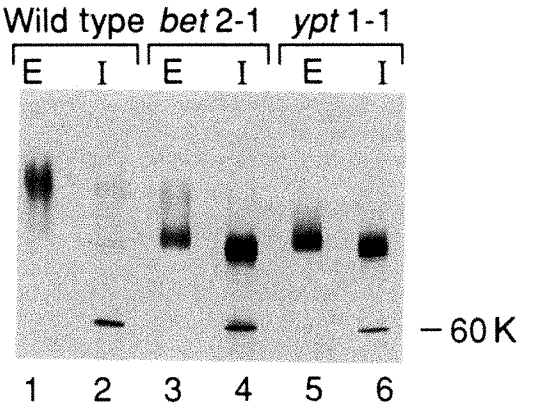

FIG. 2 The *bet2* mutant secretes an underglycosylated form of invertase. The following samples were immunoprecipitated with anti-invertase antibody and analysed on a 12.5% SDS polyacrylamide gel: lane 1, wild-type (SFNY 93; *MATα, ura3-52, his4-619*, pRB58[23]) external invertase (E); lane 2, wild-type internal invertase (I); lane 3, *bet2-1* (SFNY 92; *MATα, bet2-1, ura3-52, his4-619*, pRB58) external invertase (E); lane 4, *bet2-1* internal invertase (I); lane 5, *ypt1-1* (SFNY 94; *MATα, ypt1-1, ura3-52*, pRB58) external invertase (E); lane 6, *ypt1-1*, internal invertase (I).
METHODS. Cells were grown at 25 °C, shifted to 37 °C for 30 min, and radiolabelled at this temperature (45 min). The invertase retained within the cells and secreted was immunoprecipitated, and electrophoresed as described before[7]. Two forms of invertase, a constitutively synthesized cytoplasmic form, and a secreted form, were observed. The cytoplasmic form, of M_r 60,000 (60 k), is seen in lanes 2, 4 and 6.

a CAAX box (C, Cys; A, aliphatic residue; X, any amino acid). The CAAX box signals a series of modifications that are required for membrane binding. These events include prenylation, proteolytic processing, and carboxymethylation[14]. Although the mechanism of membrane attachment of Ypt1 and Sec4 is unknown, the carboxy-terminal CC motif of these proteins may serve a similar function[15,16]. As a first step towards determining the mechanism by which Bet2 is required for the membrane attachment of Ypt1 and Sec4, we have cloned and sequenced the gene that encodes this protein. DNA sequence analysis has revealed that the *BET2* gene encodes a hydrophilic protein of relative molecular mass ~36,500 (M_r 36.5K), which shares 56.6% similarity and 34.1% identity with Dpr1 (Ram1), an essential component of a prenyltransferase that modifies the carboxy terminus of Ras and the yeast mating pheromone **a**-factor[8]. A comparison of the Bet2 and Dpr1 (Ram1) proteins is shown in Fig. 3. *BET2* is also the same as *ORF2*, an unidentified open reading frame that encodes an essential protein of unknown function[11]. The DNA sequences of these genes are identical with one exception which results in a conserved amino-acid change at position 22 (asparagine for Bet2) of the protein. This exception may be a consequence of differences in strain backgrounds. *ORF2* and *DPR1(RAM1)* were previously reported to be homologous[11], but the significance of this homology has been unclear until now.

The mechanism by which Ypt1 and Sec4 associate with membranes has so far remained elusive. Although the presence of an isoprene group has not been identified on this class of proteins, our data implies that Bet2 is a component of a protein prenyltransferase that attaches an isoprenoid moiety to the C-terminus of Ypt1 and Sec4. Consistent with this hypothesis is the observation that the soluble pool of Sec4 is exaggerated in a mutant[17] that blocks the synthesis of mevalonic acid (P. Novick, personal communication), a precursor of all isoprenoids. As it has been proposed that Ypt1 and Sec4 regulate vesicular traffic by cycling on and off membranes[6,16,18], the modification of these proteins may be subject to turnover. This would be unlike the case for Ras where the prenylation event is irreversible[14]. Alternatively, an isoprenoid group may be stably

```
Dpr1(Ram1)    61 LQSVLEIYDDE..KNIEPALTKEFHKMYLDVAPEISLPPQMTALDASQPW 108
                 : : |.:..:.  : ||.  ||. :       ||..|..::   :
Bet2           1 MSGSLTLLKEKHIRYIESLDTKKHN.......FEYWLTEHLRLNG..... 38

Dpr1(Ram1)   109 MLYWIANSLKVMDRDWLSDDTKRKIVVKLFTISPSG...GPFGGGPGQLS 155
                   :|| ..| |:|    |.:|  |  |  |.:|..:   |:|:. | : .
Bet2          39 .IYWGLTALCVLD....SPETFVKEEVISFVLSCWDDKYGAFAPFPRHDA 83

Dpr1(Ram1)   156 HLASTYAAINALSLCDNIDGCWDRIDRKGIYQWLISLKEPNGGFKTCLE. 204
                 || .|..|:. |. :|.:|     :  |:   :|||: .|.  ..::::
Bet2          84 HLLTTLSAVQILATYDALD.....VLGKDRKVRLISFIRGNQLEDGSFQG 128

Dpr1(Ram1)   205 ..VGEVDTRGIYCALSIATLLNILTEELTEGVLNYLKNCQNYEGGFGSCP 252
                   .{{{{{ :|.{{  .:|. |:|.|:..:::: .| |::|{{ ||
Bet2         129 DRFGEVDTRFVYTALSALSILGELTSEVVDPAVDFVLKCYNFDGGFGLCP 178

Dpr1(Ram1)   253 HVDEAHGGYTFCATASLAILRSMDQINVEKLLE...WSSARQLQEERGFC 299
                   : .|.|:: .|.. :.|{{ ..:| :. :.|  |   | :.|{{.|: |:
Bet2         179 N.AESHAAQAFTCLGALAIANKLDMLSDDQLEEIGWWLCERQLPEG.GLN 226

Dpr1(Ram1)   300 GRSNKLVDGCYSFWVGGSAAILEAFGYGQCFNKHALRDYILYCLQEKEQP 349
                   ||..||.|.|||:|| :| ||:: :::   :| . | ::|{  ||:....
Bet2         227 GRPSKLPDVCYSWWVLSSLAIIGRLDW...INYEKLTEFIL.KCQDEKKG 272

Dpr1(Ram1)   350 GLRDKPGAHSDFYHTNYCLLGLAVAESSYSCTPNDSPHNIKCTPDRLIGS 399
                   |:.|:|:.. |.:|| :.: ||.                    |:|
Bet2         273 GISDRPENEVDVFHTVFGVAGLS.....................LMGY 299

Dpr1(Ram1)   400 SKLTDVNPVYGLPIENVRKIIHY 422
                   ..|..::|:|.:| . ..|: .|
Bet2         300 DNLVPIDPIYCMPKSVTSKFKKY 322
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FIG. 3 A comparison of the Bet2 and Dpr1 (Ram1) proteins. Single-letter amino-acid code used. *BET2* was cloned by complementation of the *bet2-1* growth defect, using a plasmid library containing inserts of genomic DNA inserted into the YCp50 vector[24]. A complementing fragment (~2.0 kilobases), cloned into YIp5, was shown to integrate at the *bet2* locus. To do this, we crossed a *MATa, bet2-1, ura3-52* strain to a *MATα, his4-619, ura3-52, BET2*::(*URA3, BET2*) strain and in 23 tetrads, the Ura$^+$ and Ts$^+$ phenotypes always cosegregated. A *Hin*dIII site, which were shown to be internal to the *BET2* gene by complementation studies, is contained within the open reading frame encoding the sequence shown in this figure. The 2.0-kb complementing fragment, inserted into pNRB113 (a 2 μm plasmid vector), also complemented the growth and membrane localization defects of the *bet2-1* mutant. The amino-acid sequence of *BET2* (total sequence 325 amino acids) is compared to *DPR1(RAM1)*[25] using the BESTFIT program. Identity is indicated by a line and conserved changes are marked by two dots (two corresponding bases in their codons) or one dot (one corresponding base in their codons) between the sequences. The gaps are designated by dots within a sequence.

attached to Ypt1 and Sec4 and a second element could be involved in releasing these proteins from membranes.

Bet2 seems to act on a different family of proteins from Dpr1 (Ram1), as we have found that yeast Ras is membrane-bound (Fig. 1*b*) and a-factor is biologically active in the *bet2* mutant (*MATa, bet2-1* cells mate as efficiently as wild type in a quantitative mating test). In addition, the *dpr1*(*ram1*) mutant is not appreciably suppressed by the *BET2* gene when this is introduced on a high copy number vector. The same result was also obtained in the reciprocal experiment. These findings imply that Bet2 may be a component of a novel modification pathway that recognizes carboxy-terminal consensus sequences that end in a CC motif rather than a CAAX box. As the inhibition of isoprenoid synthesis retards the growth of tumours in animal cells by preventing the attachment of Ras to membranes[19], an understanding of the specificity of different protein prenyltransferases for their substrate may be useful in the therapeutic treatment of Ras-dependent tumours[8,17,20,21]. □

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Identification *in situ* and phylogeny of uncultured bacterial endosymbionts

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THE use of Koch's technique to isolate bacteria in pure cultures has enabled thousands of bacterial species to be characterized. But for the many microorganisms that have never been cultivated, DNA amplification *in vitro* using the polymerase chain reaction is now making their genes accessible[1–3]. Here we use this technique to study bacteria of the genus *Holospora*, which live in ciliates[4] and whose phylogenetic relationship has remained unknown because they are impossible to cultivate. Species of *Holospora* are highly infectious[5–7] and live in the nuclei of their specific host cells: *H. elegans* and *H. undulata* infect micronuclei of *Paramecium caudatum*[8], whereas *H. obtusa* infects the macronucleus in other strains of the same host species[9]; *Holospora* species have a common developmental cycle[10–13]. We have amplified, cloned and sequenced gene fragments encoding ribosomal RNA of *H. obtusa*. The phylogenetic position of *H. obtusa* in the α group of *Proteobacteria* was determined by 16S rRNA sequence analysis. The sequences were then used to design species- as well as genus-specific rRNA hybridization probes, which enabled us to detect and differentiate individual cells of the endosymbionts *in situ*. The large amount of rRNA in the cells indicates a high physiological activity of the endosymbionts in the host nuclei.

Our approach to studying *H. obtusa* is summarized in the flow diagram of Fig. 1. It may be generally applicable for the study of ecosystems ill-defined by pure culture technique. Comparative rRNA sequence analysis is at present the best method for reconstructing phylogenetic relationships below the species level. We analysed the 16S rRNA of *H. obtusa* as detailed in the legend to Fig. 2. A phylogenetic affiliation was found between the newly obtained 16S rRNA sequence and the α subclass[14] of the proteobacterial phylum[15]. Within this subclass no close relative of *H. obtusa* has so far been analysed at the rRNA level. Interestingly, this phylogenetic tree reveals a moderate relationship between *H. obtusa* and the intracellular parasites *Rickettsia*. Also the mitochondrial 16S rRNAs have a common phylogenetic root with members of the α subclass of *Proteobacteria*[16].

To prove that the amplified and cloned sequences originated from the endonuclear symbionts and were not artefacts from contaminating bacteria that were used for feeding the ciliates, we performed *in situ* hybridizations with fluorescent oligonucleotide probes. This is the most direct way to link the sequences[3] to a defined morphotype[17–20]. On the basis of 16S rRNA analysis, the specific hybridization probe H16-23a (5′-TTCCACTTTCCTCTACCG-3′) was designed to differentiate the newly obtained sequence from other bacterial 16S rRNA sequences. Fluorescently labelled, rRNA-targeted oligonucleotide probes enable individual bacterial cells to be detected[18,19]. A specific probe labelled with tetramethylrhodamine was used along with a fluorescein-labelled probe complementary to all bacterial 16S rRNAs so far sequenced (5′-GCTGCCTCCCGTAGGAGT-3′)[21]. This second probe served as a control for the presence and accessibility of bacterial 16S rRNA molecules within the host paramecia.

After hybridization, epifluorescence microscopy with a fluorescein-specific filter set revealed that the bacterial 16S rRNA signature sequence was present in the food vacuoles as well as in the macro- or micronuclei (Fig. 3). But the rhodamine-labelled specific probe H16-23a only hybridized to rod-shaped cells in the macro- or micronuclei of infected paramecia (Fig. 3). As many as several thousand *H. obtusa* cells were densely packed into the single macronucleus (up to 80 μm in diameter), and several hundreds to thousands of *H. elegans* or *H. undulata* cells filled the micronuclei (one to three per cell with a diameter of 20 to 50 μm). There were no suitable target sites within the *H. obtusa* 16S rRNA sequence for probes to distinguish the three closely related species from one another. We therefore sequenced a highly variable part of the 23S rDNA of *H. obtusa* and constructed a species-specific probe (Hobt56a 5′-AACACCCTACTTCTTTCG-3′). Applying this probe as described could differentiate between micro- and macronuclear endosymbionts in the host cells so that only *H. obtusa* was identified. This clearly demonstrated that we had amplified,

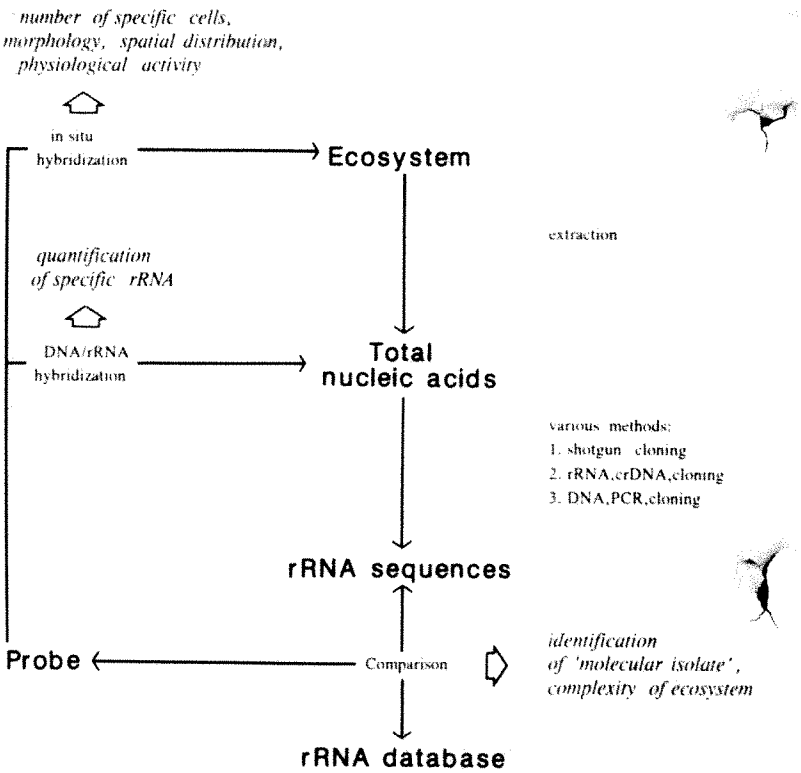


FIG. 1 General approach to characterize a mixed biological sample by rRNA sequence analysis and subsequent hybridization with rRNA-targeted specific probes without prior cultivation. Categories of information that are obtained are indicated in italics. Comparative analysis of the rRNA sequences provides an estimate for the genetic diversity and consequently the complexity of the ecosystem under investigation. The phylogenetic position of the cloned rRNA fragment can be derived. Two subsequent DNA–rRNA hybridizations with a universal probe and a specific probe reveal the relative amount of a specific rRNA in the total extracted nucleic acids. Hybridization *in situ* with a specific probe provides information about cell numbers, morphology, spatial distribution, and qualitative estimates of the physiological activity of single cells.

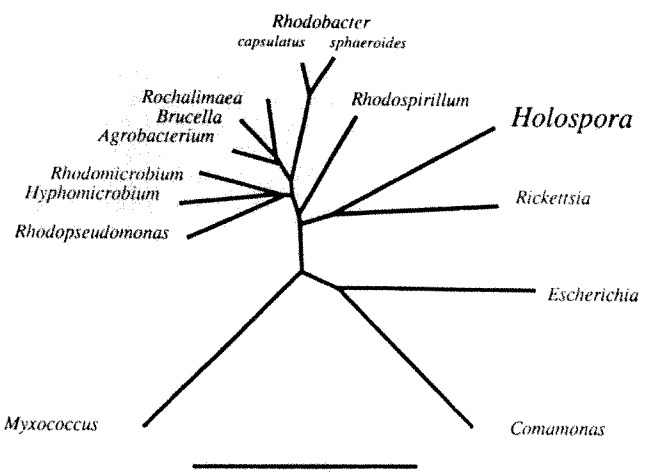
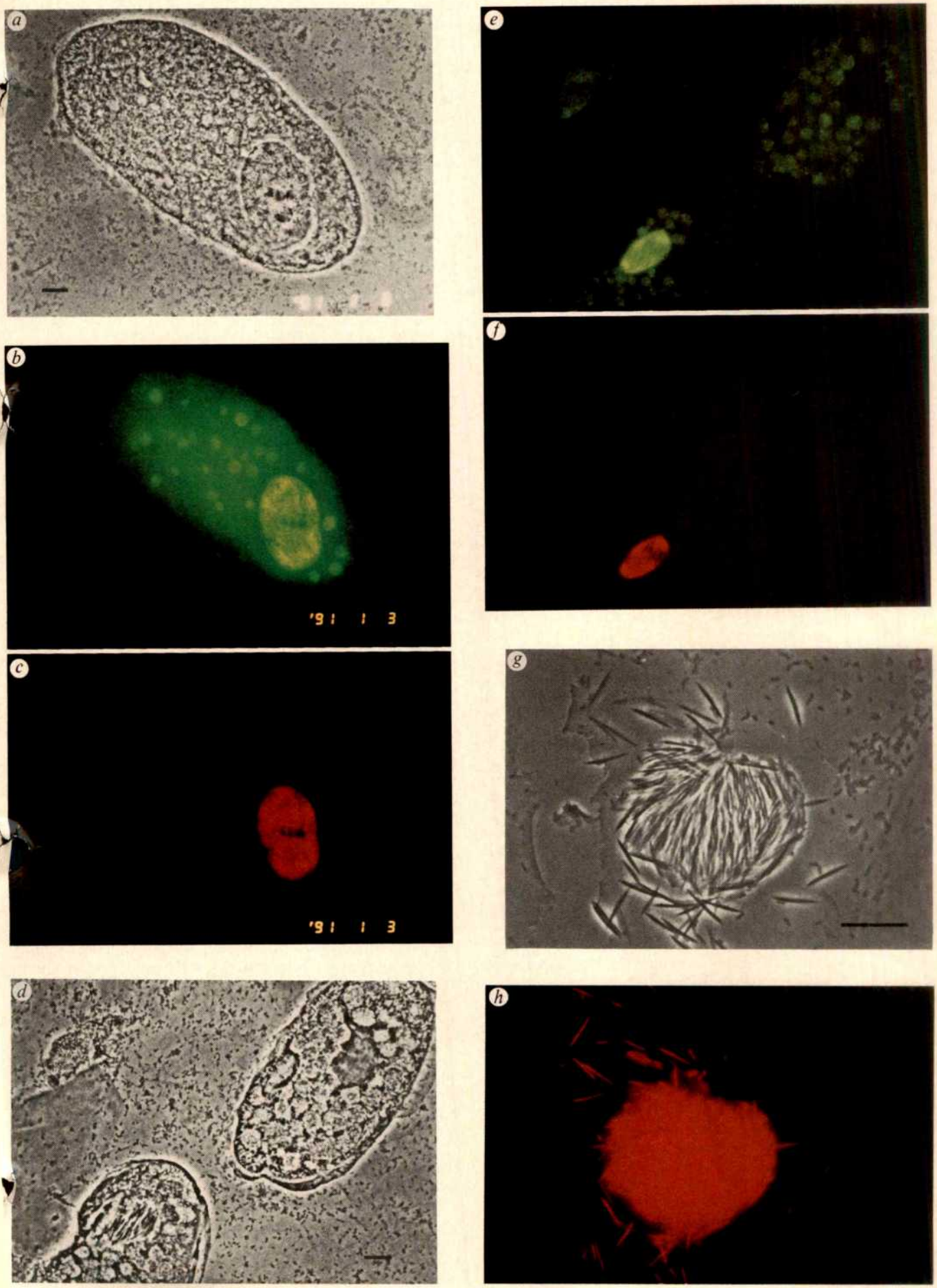


FIG. 2 16S rRNA-based phylogenetic tree showing the relationship of *H. obtusa*. This tree bases on a matrix of phylogenetic distances[23] which was calculated from aligned 16S rRNA sequences including only alignment positions that are invariant in at least 50% of the entire set of sequences. The tree was reconstructed using the neighbour-joining method of Saitou and Nei[24]. The reference sequences were from *Agrobacterium tumefaciens*[25] (α), *Brucella abortus*[25] (α), *Comamonas testosteroni*[25] (formerly *Pseudomonas*) (β), *Escherichia coli*[25] (τ), *Hyphomicrobium vulgare*[25] (α), *Myxococcus xanthus*[25] (δ), *Rickettsia rickettsii*[26] (α), *Rochalimaea quintana*[25] (α), *Rhodobacter capsulatus* EMBL M34129 (α), *Rhodobacter sphaeroides*[26] (α), *Rhodopseudomonas acidophila* EMBL M34128 (α), *Rhodospirillum rubrum* EMBL M32020 (α). Greek letters indicate the corresponding subclass of *Proteobacteria*. The bar indicates 0.05 Knuc (phylogenetic distance)[23]. The 16S rRNA sequence of *H. obtusa* was submitted to EMBL.

METHODS. For enrichment of genomic DNA, infectious forms of endosymbiotic *Holospora* from homogenates of infected *Paramecium caudatum* (ATCC 50009) were pelleted to the bottom of a 90% Percoll cushion[12]. Lysis of the bacteria and DNA purification were as described[27]. From DNA prepared from an enrichment of *H. obtusa*, two overlapping DNA fragments were amplified and cloned as pHobt161 and pHobt162. pHobt161 contains rDNA homologous to *Escherichia coli* 16S rRNA positions 7 to 1,510, whereas pHobt162 contains rDNA corresponding to *E. coli* 16S rRNA positions 1,396 to 1,542, an intergeneric spacer and rDNA homologous to *E. coli* 23S rRNA positions 1 to 130. The nucleotide sequences of the primer pairs used were: 5′-AGAGTTTGATYMTGGCTC-3′/5′-GGTTACCTTGTTACGACTT-3′ and 5′-CACCGCCCGTCACACC-3′/5′-GGGTTYCCCCATTCGG-3′. The 16S rDNA nucleotide sequence was determined by using the chain termination technique[28]. The sequence was aligned with about 300 bacterial 16S rRNA sequences[29].

FIG. 3 (Opposite) Detection and differentiation *in situ* of *H. obtusa* and *H. elegans* in *Paramecium caudatum*. Bars, 20 μm. *a–c*, Identical microscopic fields after simultaneous hybridization of *H. obtusa* infected *Paramecium caudatum* cells with the bacterial probe labelled with fluorescein (*b*) and the probe labelled with tetramethylrhodamine specific for *H. obtusa* targeting 23S rRNA (Hobt56a, *c*). *d–f*, Identical microscopic fields showing a *P. caudatum* cell infected with *H. elegans* hybridized with the bacterial probe labelled with fluorescein (*e*) and the probe labelled with tetramethylrhodamine and specific for *Holospora* (H16-23a, *f*). *g, h*, Remains of a micronucleus of *P. caudatum* infected with *H. elegans* and hybridized with tetramethylrhodamine-labelled H16-23a (*h*). *a, d* and *g*, Phase contrast micrographs; *b, e*, epifluorescence micrographs using the Zeiss (Oberkochem, Germany) filter set 09, with an additional short pass 560-nm filter to excite selectively the fluorescein-labelled bacterial probe (exposure time of 10 s); *c, f* and *h* are epifluorescence micrographs obtained with the Zeiss filter set 15 which selectively excites the tetramethylrhodamine-labelled probes (exposure time of 2 s).

METHODS. Samples of infected *P. caudatum* cells and prey bacteria were separated from the substrate by centrifugation at 12,000*g* for 1 min. After resuspension in 0.05% agarose solution, cells were attached to glass slides by air drying. Cells were fixed in paraformaldehyde and ethanol as described[21]. Fluorescent oligonucleotide probes where synthesized following standard protocols[20]. Hybridization *in situ* was performed at 45 °C in a buffer containing 0.9 M NaCl, 20 mM Tris-HCl and 0.01% SDS, with a probe concentration of 5 ng μl^{-1} for 1 h. The hybridization mixture was removed and the slide washed for 20 min in the same buffer at 48 °C. Salts were removed with distilled water, the slides were air-dried and viewed immediately after embedding in a glycerol-containing mountant (Citifluor, UK).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cloned and sequenced parts of the 16S and 23S rDNA molecules of *H. obtusa*.

Using rRNA-targeted oligonucleotide probes *in situ*, even more information of ecological importance can be retrieved. The amount of bound probe is directly correlated to the cellular rRNA content, which itself is a measure of physiological activity[18]. By this criterion most of the endosymbionts are highly active in their habitat, because hybridization of the bacterial probe is as strong (or stronger) with endonuclear *Holospora* cells as it is with surrounding *Enterobacter aerogenes* which are added as feed from a logarithmic culture.

We have described an approach that allows a definitive correlation to be made between an unknown sequence obtained from a complex environment, and cells living in that environment. The techniques should help microbial taxonomists and ecologists learn more about organisms that have so far proved impossible to culture. This should be particularly useful, as it is thought that only a small part of the microbial diversity has so far been discovered and studied[1,2,22]. □

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Key residues involved in calcium-binding motifs in EGF-like domains

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MANY extracellular proteins with diverse functions contain domains similar to epidermal growth factor (EGF), a number of which have a consensus Asp/Asn, Asp/Asn, Asp*/Asn*, Tyr/Phe (where the asterisk denotes a β-hydroxylated residue)[1]. These include the coagulation factors IX and X, proteins with two EGF-like domains, the first of which contains the consensus residues[2]. The first EGF-like domain of human factor IX contains a calcium-binding site, which is believed to be responsible for one of the high-affinity sites detected in this protein[3]. Similar results have been obtained for bovine factor X[4]. We have now used protein engineering and ^{1}H-NMR techniques to investigate the importance of individual consensus residues for ligand binding. Measurement of a calcium-dependent Tyr 69 shift[3] in the isolated first EGF-like domain from human factor IX demonstrates that Asp 47, Asp 49, and Asp 64 are directly involved in this binding. Gln 50, whose importance has previously been overlooked, is also involved in this binding. Two mutations[5] in this domain, Asp 47 → Glu, and Asp 64 → Asn, present in patients with haemophilia B, reduce calcium binding to the domain >4-fold and >1,000-fold, respectively. Furthermore, the defective calcium binding of Asn 64 can be partially rescued by the compensatory mutation Gln 50 → Glu. This latter mutation, when introduced singly more than doubles the affinity of the domain for calcium. This study thus defines residues involved in a new type of calcium-binding site and provides strong circumstantial evidence for calcium-binding motifs in many extracellular proteins, including the developmentally important proteins of *Drosophila*, notch, delta and crumbs[1,6–8].

The sequence of the first EGF-like domain from human factor IX is shown in Fig. 1*a*. A structural model of this domain, based on the ^{1}H-NMR solution structure of human EGF[9] (Fig. 1*b*), shows the predicted arrangement and the proximity of the consensus residues Asp 47, Asp 49, Asp 64 and Tyr 69 to one another. To test the importance of each carboxylate for calcium binding, mutations were introduced into the domain (see Table 1). These include two mutations identified in haemophilia B patients (referred to as Oxford d1 and d3)[5] which have the point mutations Asp 64 → Asn and Asp 47 → Glu respectively, and two mutations previously studied in genetically engineered factor IX proteins[1], Asp 49 → Glu and Asp 64 → Lys, which have not been detected in patients. Mutant domains (residues 46–84 of human factor IX) were expressed using a yeast secretion system[3] (for details of cloning procedures see legend to Table 1), refolded and purified by HPLC. Each mutant domain was analysed by ^{1}H-NMR and its spectrum compared with the wild-type domain to determine whether the introduced mutation had disrupted structure. Figure 2 shows the one-dimensional NMR spectrum at pH 7.4 of the aromatic region of four of the mutant domains compared with the wild-type domain. All show striking similarity to the wild-type profile, indicating that the introduced mutations have not disrupted the overall domain structure—a prerequisite for these calcium binding studies. All other mutant domains tested showed similar profiles. Calcium (as $CaCl_2$) was then titrated into each protein sample in the absence of other ions and the movement of the calcium-dependent Tyr 69 resonance (arrowed in Fig. 2) recorded. A subsequent set of calcium titrations was performed at constant ionic strength ($I = 0.15$, equivalent to physiological ionic strength) to exclude this as a variable affecting calcium binding. The change in chemical shift of the Tyr 69 resonance was plotted against free calcium concentration to obtain a dissociation constant (Fig. 3*a*). Dissociation constant (K_d) values are summarized in Table 1.

In mutants Asp 47 → Glu, Asp 49 → Glu and Asp-64 → Glu, or Lys, or Asn, there is a large reduction in the affinity (increase in K_d) of the domain for calcium, at both variable and constant ionic strength. Asp 49 and Asp 64 seem to be more important ligands than Asp 47 as in the examples where conservative

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mutations (Asp → Glu) have been introduced at each of the three carboxylate residues, the affinity of these mutants for calcium is most severely affected (see Table 1). In addition, there are no marked pH effects on the K_d for these mutants over the range measured (pH 6.5–8.5, Fig. 3*b*). This argues against the reduced affinity being caused by an indirect effect of the mutation on the pH optimum for calcium binding. Two other mutations introduced at Asp 64 (Asp → Asn, Asp → Lys) seem to have an even greater inhibitory effect on calcium binding. No binding was detected in the case of the Asp 64 → Lys mutation and only a slight movement of the Tyr 69 resonance was detected at 250 mM Ca^{2+} for the Asp 64 → Asn mutation. In summary, these data establish the critical importance of the carboxylate residues Asp 47, 49 and 64 for calcium binding.

The virtual absence of calcium binding to the Asp 64 → Asn mutant domain is of interest as many examples of EGF-like domains are known with an Asn residue in the equivalent position to Asp 64 of factor IX[1]. Closer examination of these EGF-like domain sequences, however, reveals that, in general, Asp at position 64 is associated with either Gln (type I consensus, see Fig. 1*a*) or Glu (type III consensus) at position 50, whereas Asn is associated with Glu rather than Gln (type II consensus). This suggested that the residue at position 50 in factor IX may be important in calcium binding. Therefore we constructed a double mutant Asp 64 → Asn, Gln 50 → Glu, as well as the single mutant Gln 50 → Glu, to test if we could rescue calcium binding which is absent in the mutant Asp 64 → Asn. These represent the type II and type III combinations of consensus residues (see Fig. 1*a*) within a factor IX EGF-domain background. In each we could demonstrate improved calcium binding at constant ionic strength (Asp 64 → Asn, Gln 50 → Glu, $K_d \sim 10$ mM; Gln 50 → Glu, $K_d = 0.5$ mM; Table 1 and Fig. 3*a*). These mutants, together with the wild-type domain, thus indicate that all three variations of the consensus sequence (type I, II, III) in a factor IX background support calcium binding. Recent two-dimensional ^{1}H-NMR structural analysis of the first EGF-like domain from factor IX has confirmed that Gln 50 is close in space to Tyr 69 and thus in the vicinity of the calcium-binding region[10]. The rescue of calcium binding to the Asp 64 → Asn mutant domain by the introduction of a second mutation Gln 50 → Glu was pH-independent (over the range measured, pH 6.5–8.5; Fig. 3*b*). This provides strong evidence for the participation of Glu 50 in direct ligand binding in this domain. In the case of the single mutant Gln 50 → Glu domain we cannot, however, rule out the possibility that the introduction of an additional ionizable group has indirectly increased affinity by

TABLE 1 Values of K_d for Ca^{2+} binding to mutant domains

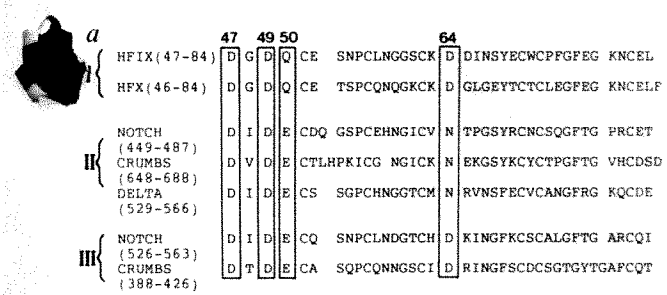
Mutation in FIX EGF domain	K_d at pH 7.4 (mM) I = variable†	I = 0.15	Specific activity* Intact factor IX (%)
Wild type	0.25	1.8	100
Asp 47 → Glu (Oxford d3)	2.5	8.9	1[5]
Asp 49 → Glu	4.0	67	8[1]
Asp 64 → Glu	4.0	105	—
Asp 64 → Lys	Undetectable	—	2[1]
Asp 64 → Asn (Oxford d1)	>250	—	3[5]
Asp 64 → Asn, Gln 50 → Glu	1.5	10.2	—
Gln 50 → Glu	<0.1‡	0.5	—

Mutations were introduced into the factor IX EGF-like domain coding sequence by standard protein engineering techniques[16] or by the polymerase chain reaction procedure[17]. In the latter case oligonucleotides were used to amplify a region of genomic DNA corresponding to nucleotides 10,391–10,504 of the factor IX sequence[18]. Amplifications were performed on a Cetus DNA thermal cycler using an initial denaturation step of 95 °C for 7 min, followed by 30 cycles of 91 °C for 1 min, 55 °C for 1 min, 70 °C for 3 min. Buffer and enzyme additions were as described previously[17]. Amplified DNA was directly sequenced before cloning into the yeast expression vector[19]. Details of the yeast secretion method used to express the domains have been reported elsewhere[3]. Mutant domains (0.5–2 mg) were refolded before analysis[20]. N-terminal sequence analysis of the domains by Edman degradation confirmed that all contained the correct mutations. The domains produced by the yeast biosynthetic route did not contain the post-translational modifications present in human factor IX, specifically a β-hydroxyaspartic acid at position 64[21], and an *O*-linked carbohydrate modification on Ser 53[22]; however, neither of these modifications is necessary for calcium binding to the wild-type domain[3]. —, Not done.

* Specific activity is defined as clotting activity/antigen where factor IX antigen and clotting activities are expressed as a percentage of that antigen or clotting activity found in normal human plasma.

† The K_d values obtained when I = variable (in the absence of NaCl) should be regarded as apparent K_d values.

‡ 0.1 mM represents the lower limit of our experimental accuracy for the quantitative determination of K_d.



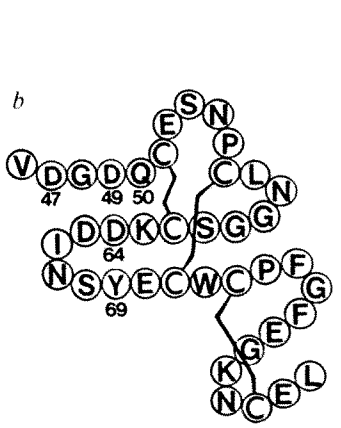


FIG. 1 *a*, Sequence comparison of some EGF-like domains with the Asp/Asn, Asp/Asn, Asp*/Asn*, Tyr/Phe. For a more detailed list Residues Asp 47, Asp 49, Gln 50 and Asp 64 (numbering as in which are implicated in calcium binding, are boxed. Three variat consensus are shown. Type I: Asp, Asp, Gln, Asp (for example hu IX, human factor X); type II: Asp, Asp, Glu, Asn (for example notch crumbs 648–688, delta 529–566); type III: Asp, Asp, Glu, Asp (fo notch 526–563, crumbs 388–426). Sequences for notch, delta, a were obtained from published data[6–8]. *b*, The first EGF-like dom human factor IX (residues 46–84) modelled on the solution stru human EGF[9]. Residues referred to in the text are numbered. Singl amino-acid code is used.

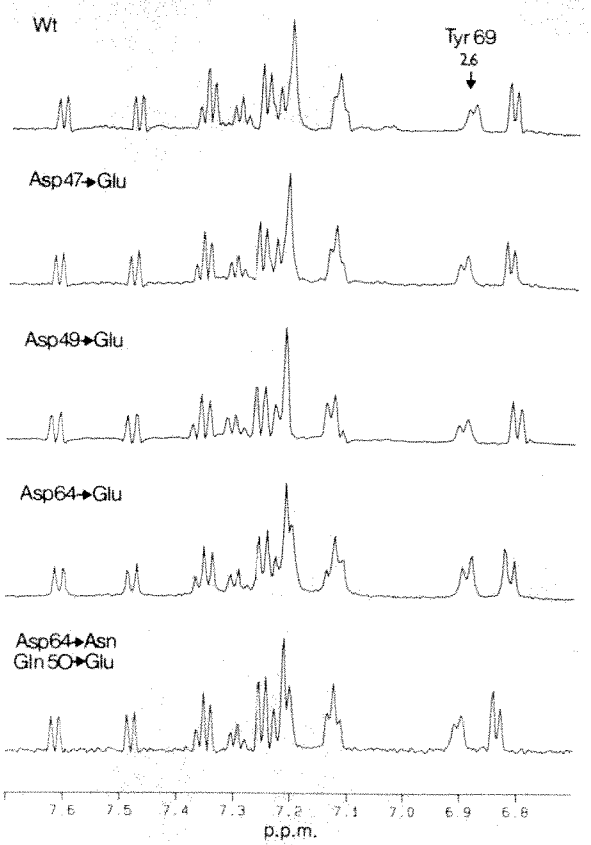
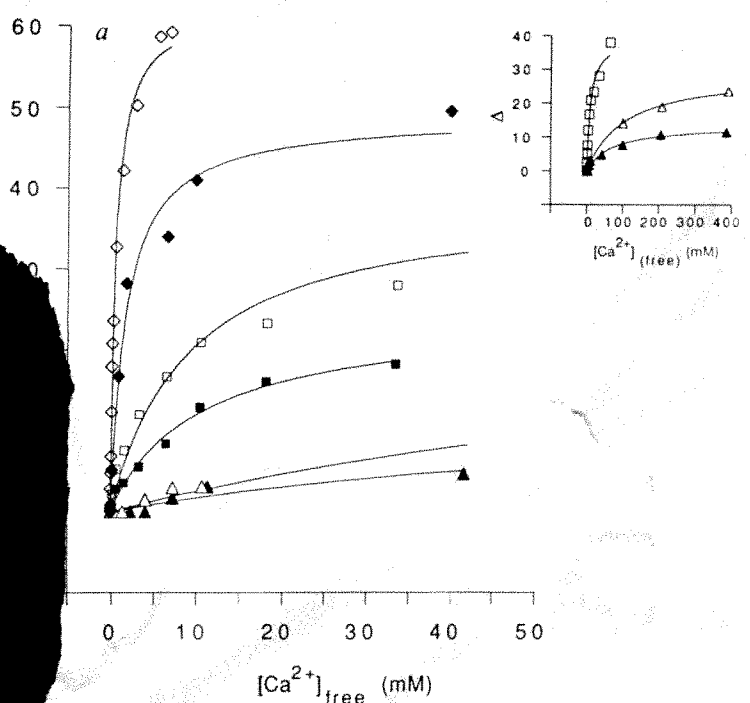
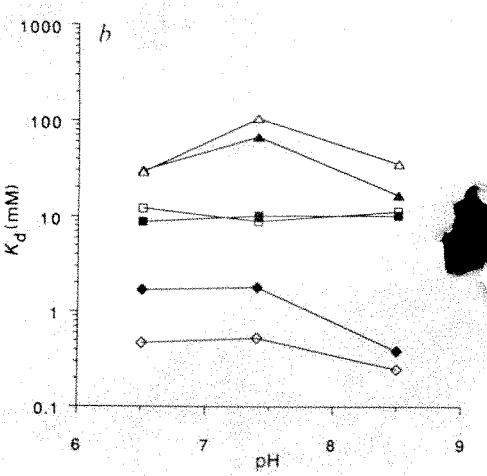


FIG. 2 A comparison of one-dimensional ^{1}H-NMR spectra of wild-type (Wt) and mutant factor IX EGF-domains at pH 7.4, 303 K, in D_2O, in the absence of Ca^{2+} and other ions showing the aromatic region of the spectrum. Spectra were collected on a Bruker AM500 MHz spectrometer. Assignment of resonances in this region have been described previously[3]. The Tyr 69, 2,6 proton resonance used to measure calcium binding to the domain is indicated with an arrow.





The change in chemical shift (Hz) of the Tyr 69, 2,6 proton resonance H7.4, 303 K and constant ionic strength (I = 0.15) plotted against cium. Gln 50 → Glu (◇), wild type (◆), Asp 47 → Glu (□), Asp 64 → Asn, → Glu (■), Asp 49 → Glu (▲), Asp 64 → Glu (△). Protein samples were lved in D_2O and the pH adjusted to 7.4. For calcium titrations carried at constant ionic strength, NaCl was added to the protein sample to e a final concentration of 0.15 M (I = 0.15), which approximates to physio- gical ionic strength. Stock $CaCl_2$ (at pH 7.4) was adjusted with NaCl to give a total ionic strength of 0.15 using the formula $I = \frac{1}{2}\Sigma cZ^2$, before addition to the sample. Curves were fitted according to the equation $\Delta = \Delta_0[Ca_{free}]/(K_d + [Ca_{free}])$ by standard linear regression analysis. Free calcium was calculated from the equation $[Ca_{free}] = [Ca_{total}] - \Delta/\Delta_0[protein]$ (assuming one binding site), where Δ/Δ_0 is the fractional change in the chemical shift of the Tyr 69 resonance, in order to correct for the relatively high amounts of protein used in the experiment. For mutants Asp 64 → Glu, Asp 49 → Glu, Asp 47 → Glu, data points obtained at ionic strength >0.15 were included for the calculation of K_d values. The inset graph shows the complete calcium titration data for these mutants. *b*, The pH dependence of calcium binding to factor IX EGF-like domains. Calcium titrations were carried out at constant ionic strength (I = 0.15) at pH 6.5, 7.4, 8.5, as detailed above; symbols as for Fig. 3*a*.

altering the pH optimum for calcium binding, as the wild type binds calcium with a similar affinity to Gln 50→Glu at pH 8.5 (Fig. 3*b*). Despite this, our binding data, together with the observed sequence conservation discussed above, clearly involve residue 50, either directly or indirectly, with calcium binding.

Our data suggest that the reduced clotting activities in mutant factor IX samples, and thus the phenotypic effect of mutations in the haemophilia B patients Oxford d1 and d3, are due to reduced calcium binding to the isolated domain (Table 1). It is unlikely that the reduction in clotting activity is due to gross perturbation of the EGF domain as NMR analysis of the isolated domains showed them to be virtually identical to the wild type in the absence of calcium (Fig. 2).

The two reported high-affinity calcium-binding sites in Gla-domainless factor IX, measured in the presence of 0.1 M NaCl, have dissociation constants of ~50 μM[11]. We have previously argued that the K_d observed for the isolated domain in NMR experiments corresponds to one of the calcium-binding sites in Gla-domainless factor IX, although the binding to the isolated domain is weaker[3]. Similar results have been obtained for the isolated bovine factor X domain[4]. Additional contacts outside the first EGF-like domain presumably increase the affinity of the site.

The precise role of calcium in the EGF-like domain is unclear. Calcium bound to the domain may stabilize a conformation required for protein–protein interactions, a common feature of proteins containing these domains[1,12–15]. More speculatively, if two proteins with these domains were required to interact, then calcium ions might bridge two EGF-like domains, with specificity being controlled by additional contacts outside this region. It has recently been demonstrated that notch and delta, two proteins involved in the neurogenic developmental pathway of *Drosophila*, directly interact in a calcium-dependent manner[12]. This is of interest as notch has 36 EGF-like domains and delta has 9, of which 6 and 1, respectively, contain calcium-binding sequences of the type studied here[6,7]. Although direct interaction through these domains has not been demonstrated, the fact that the interaction is calcium-dependent is highly suggestive. Furthermore, the *Drosophila* crumbs protein has 5 out of 30 EGF-like domains with calcium-binding motifs demonstrated in this study[8]. It is possible that calcium-dependent interactions form an integral part in the role of this protein in epithelial organization. □

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Controlling laboratory vibrations

Patrick Wong

Tabletop vibrations, when not controlled, can adversely affect sensitive instrumentation. The sources of these laboratory vibrations and various vibration control methods are discussed.

CRITICAL biomedical experiments can be seriously affected by vibrations. When tabletop vibrations are not controlled, sensitive instrumentation is affected and research results are often seriously degraded. And, in applications such as electrophysiology, microinjection and cell manipulation, tabletop vibrations can cause complete failure of the experiment. For example, in precision work, such as the study of cell signalling in living brain slices conducted by Stephen Smith at Stanford University, even micron-scale relative motion between tabletop components can mean experimental failure. These minute vibrations can shake electrodes out of cells or they can cause mechanical tip displacement, which may rupture cells and make it necessary to repeat the experiment. As a result, it has become important to design worktables for the biomedical laboratory that are capable of providing a level of vibration control that allows ultraprecision.

There are five primary sources of disturbances that affect mechanical alignment in precision worktables. These include: ground or floor vibrational inputs; airborne vibrations (acoustic); vibrations generated by equipment or apparatus within the system; load changes of quasi-static forces that act on the system; and thermal changes such as heat source or ambient temperature changes. Although no vibration control system can completely eliminate every source of instability, one fact is clear: the better the vibration control, the better the equipment performs.

What are the alternatives?

The key to minimizing the effect of vibrations on sensitive equipment is to create a worktable situation where all parts of the system are moving together, rather than shifting around relative to each other. To accomplish this stability, the mounting surface of the worktable must have a high degree of rigidity and it must be isolated from environmental vibrations. Although a variety of crude vibration isolation methods (that is, the use of tennis balls positioned under cement blocks) have been tried in many laboratories, three main vibration control technologies have become dominant: (1) granite blocks, (2) aluminium honeycomb core tables with broadband, visco-elastic dampers, and (3) steel honeycomb cores with tuned-spring mass hydraulic dampers.

As critical experiments such as video microscopy, electrophysiology and ultramicrotomy require interferometric accuracy from all instruments used on the tabletop, vibration control worktables must be designed to meet a demanding set of performance requirements. Granite slabs, the conventional solution for isolating vibrations, have been tried in some laboratories. However, they are bulky and have many drawbacks in crowded biomedical laboratories. For example, they can weigh a ton or more, making structural support and size major obstacles. They are also limited in their ability to provide the extremely high level of vibration control needed to perform delicate experiments, such as the study of living brain slices mentioned earlier, as they are easily excited by both acoustic and mechanically transmitted vibrations.

While it may seem difficult to induce vibrations in a block weighing thousands of pounds, granite cannot match the dynamic rigidity (the ability to damp, or resist the deformation that results from internal and external vibrational inputs) of a far lighter damped steel-honeycomb construction. Mass that does not contribute to stiffness only decreases the resonant frequencies of a structure, thus increasing displacements. Granite blocks subjected to vibrations have pronounced resonance peaks. Also, as the internal damping of granite is low, the material continues to vibrate, or 'ring', long after the stimulus ceases. These micron-scale motions reduce the accuracy, precision and repeatability of critical experiments.

The use of lightweight honeycomb structures for worktables offers a greater level of vibration control as well as a much higher level of flexibility for the biomedical laboratory where space and transportability are important considerations. The core structure of a honeycomb table provides a high strength-to-weight ratio and a stiffer, more stable platform. The low weight of honeycomb structures keeps the resonant frequency high — minimizing large-amplitude, low-frequency displacements. Additionally, internal damping dissipates the vibrational energy of the tabletop by converting small amplitude mechanical vibrations to heat. This lowers resonant motion peaks across the entire vibration frequency spectrum. Because every table has dominant bending and torsional modes (each of which has a characteristic frequency and table deformation shape), optimal damping of these resonant modes vastly improves the stability of the table. Two primary technologies are used for damping: broadband, visco-elastic dampers and tuned spring mass hydraulic dampers.

Tuning out resonances

The broadband, visco-elastic dampers indiscriminately absorb a moderate amount of energy over a wide range of frequencies. But, as they are not tuned to damp dominant table resonances, they are much less effective than tuned dampers. Additionally, the damping characteristic of the visco-elastic material changes over time, making future vibration control performance unpredictable.

The use of multiple tuned dampers, each individually tuned to minimize table motion at specific resonance modes, is a much more effective solution (see Fig. 1). Tuned dampers concentrate damping where it is needed most, at the frequencies of the dominant resonance modes. Because broadband dampers are designed to provide moderate damping over a wide range of frequencies, they are not as effective at damping the dominant modes of vibration.

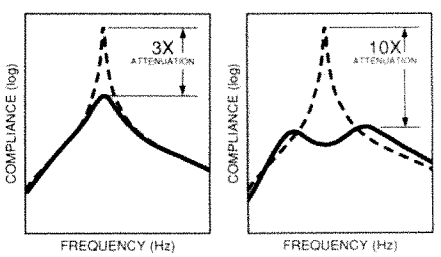

FIG. 1 Tuned dampers (left) concentrate damping at the frequencies of dominant resonance modes. As broadband dampers (right) are designed to provide moderate damping over a wide range of frequencies, they are not as effective at damping the dominant modes of table vibration.

Although honeycomb cores may be constructed from either steel or aluminium, steel typically provides greater vibration control. The comparison is best illustrated by looking at compliance curves (Fig. 2). Basically, compliance curves show the displacement amplitude of a point on a body per unit of impulse force applied. The greater the compliance, the more easily the structure moves as a result of applied force. The compliance curve for the steel tabletop in figure 2 demonstrates that the table bending with the same force is about 1.8×10^{-5} mm N^{-1}, or roughly a factor of 11 more rigid than the standard aluminium honeycomb tabletop of the same size.

Isolating floor vibrations

Most biomedical laboratories are plagued with floorborne vibrations resulting from, for example, elevators, compressors, air con-

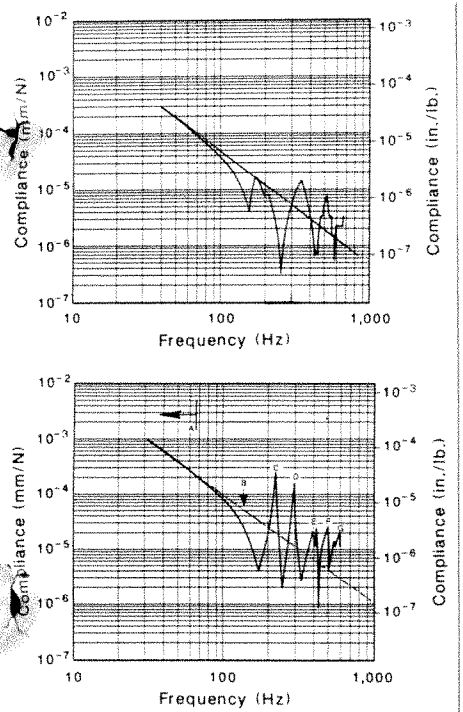

FIG. 2 The compliance curve for the steel tabletop (top) shows that the table bending with the same force is a factor of 11 more rigid than the standard alumimium tabletop (bottom) of the same size.

ditioners, and transformers, causing the table structure to resonate. As a result, the tabletop must be isolated from these 'resonance' vibrations as well as other tabletop vibrations. Of the main methods of isolating floorborne vibrations, pneumatic isolators offer the greatest level of vibration control. This is because they combine the fast 'roll-off' of the simple harmonic oscillator at vibration frequencies above isolator resonance and the low amplification of the damped harmonic oscillator near resonance.

In a pneumatic isolation system, the isolated mass is supported by a piston that rests on a flexible rolling diaphragm. Under the diaphragm are two pressurized air chambers connected by a small orifice. Air moves from the upper to the lower chamber through the orifice, dissipating energy and reducing amplification at the isolator's natural frequency.

Vibration control systems that are capable of interferometric accuracy are not only an important consideration in the biomedical laboratory, they are essential. When conducting experiments, that require ultraprecision, the floor, air and other room vibrations found in a typical laboratory can cause research delays or even experimental failure. As a result, the lightweight steel-honeycomb core worktable with pneumatic isolators has emerged as a useful vibration control device for the biomedical laboratory. □

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Made to measure

A method for the dynamic *in situ* measurement of cell growth and viability, a portable photosynthesis analysis system, and software for collecting and analysing behavioural data — new aids for quantitative analysis.

ADAM Hilger, the publishing arm of the Institute of Physics has launched two new **books** in the series on Measurement Science and Technology *(Reader Service No. 101)*. The first of the two books, entitled *SQUIDS, the Josephson Effects and Superconducting Electronics* by J.C. Gallop, outlines the development of superconducting electronics, and describes the fundamental principles, superconducting devices and their applications. In addition, practical tips and hints are provided for researchers using SQUIDs in their studies. The book costs £45 (UK). The second book is entitled *Uncertainty, Calibration and Probability: The Statistics of Scientific and Industrial Management* by C.F. Dietrich. Given that all measurements are subject to error, no quantity can be known exactly and, hence, any measurement has a probability of lying within a certain range, the IOP says this £90 (UK) book gives a comprehensive treatment of the statistics and methods of estimating these calibration uncertainties.

The CellStat system from ONCOtherapeutics provides a method of continuously **monitoring cell proliferation and viability in culture**, without disrupting important homotypic and heterotypic cell–cell relationships *(Reader Service No. 102)*. CellStat technology uses a biosensor lid that interfaces with a standard 24-well tissue culture plate to measure minute electrochemical changes in cultured cells. The conductivity of the media, which is affected by cell density and viability, is monitored and the signals integrated by computer. The company says the CellStat system has been used to monitor the growth of normal and neoplastic cell types, including a variety of human colon cancer cells, and to assess the cytostatic and cytotoxic effects of the chemotherapeutic agents 5-fluorouracil and methotrexate. Cell death due to cytotoxic agents was dynamically and continuously monitored and the results depicted graphically, yielding specific dose-response curves for each cell type. The basic unit costs $37,500 (US) and includes signal processing PC, modem, logic system, measurement system, enhanced graphics display unit, data acquisition, data analysis and data display software. The BioProbe lids cost $50 (US) each.

A fully portable, battery-operated system for the **measurement of photosynthesis and transpiration** has been developed by the Analytical Development Company *(Reader Service No. 103)*. The LCA-2 System, which consists of an infrared analyser, air-supply unit, data logger and leaf chamber, provides information on photosynthetic rate, stomatal conductance, transpiration rate, humidity and CO_2 concentration. The leaf chamber incorporates sensors to measure humidity, temperature and photosynthetic active radiation. Four standard leaf-chamber designs are available, each manufactured for different plant species.

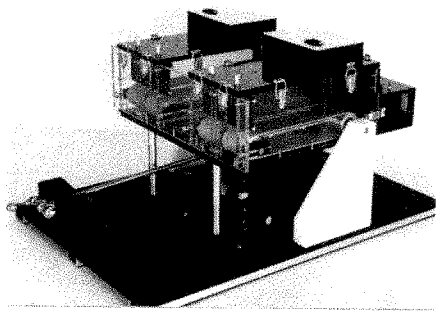
Research aids

Noldus Information Technology has developed Version 2.0 of the Observer **software package**, which turns an IBM PC or laptop computer into a powerful tool for recording the frequency and duration of behavioural events *(Reader Service No. 104)*. It can also program various handheld computers for use as event recorders in the field.

Observational research software.

When using Observer, the keys of the computer keyboard are redefined to represent events (for example, w = walking, s = sitting). As many as 92 keys can be designated as events, says Noldus. Events can have one or two 'modifiers' to indicate the scope of an event, which can be useful when studying social interactions. The program also allows grouping of events in classes and distinction between mutually exclusive versus non-exclusive, and duration versus frequency events. In addition, up to 40 independent variables (for example, experimental conditions, environmental variables) can be specified. The Observer can create output files in a format that is suitable for importation into spreadsheet or statistics programs. The software is supplied either as a Base Package for IBMcompatible PCs, or as various Support Packages for non-IBM-compatible computers.

The four-channel, **small animal exercise treadmill** from Columbus Instruments allows for the simultaneous measurement of oxygen consumption of four mice or small rats under variable exercise conditions



Four-animal exercise treadmill.

(Reader Service No. 105). The treadmill features four separate airtight animal compartments, each of which is equipped with its own belt and air mixing fan. In order to measure O_2 consumption, air from the compartments is circulated through O_2 and CO_2 sensors. Both the speed and inclination of the treadmill can be adjusted, says Columbus. In addition, the treadmill is fitted with either an electrical or air-puff stimulus to stimulate the animals to exercise.

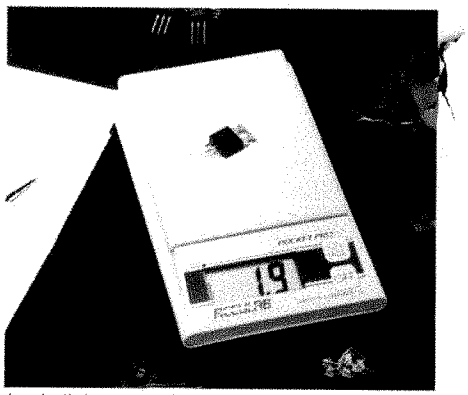
Counting devices

The multidetector 1450 MicroBeta **liquid scintillation counter** from Pharmacia is designed to cut down on both consumables and radioactive waste: samples are counted directly in microtitre plates and so the need for vials is eliminated *(Reader Service No. 106).* The six detectors of the 1450 MicroBeta are designed to provide high throughput with beta- or gamma-labelled samples. The MicroBeta has a sample capacity of up to 1,536 for 96-well microtitre plates and 384 for 24-well plates. The counter, which provides the user with an automatic d.p.m. program, chemiluminescence correction and built-in data management software, can be interfaced with automatic sample preparation systems.

An upgraded version of the Protos **colony counter** from Ai Cambridge was launched earlier this month with a host of additional functions designed to make life easier for the microbiologist *(Reader Service No. 107).* Measurements can be made on a variety of colonies, including bacteria, cells and plaques, and on media such as opaque and transparent agars, membrane filters and agarose gels. Protos can count objects of any shape, says Ai Cambridge, provided that there is a detectable difference in contrast between the sample and the background. In addition, users can now vary the colour coding of the displayed image to assist in identification and detection. Algorithms for debris counting have been incorporated and user-defined frames can be set up to scan areas not overgrown by spreading colonies, with the software allowing extrapolation to the full frame. A macro facility is available for researchers carrying out repetitive tasks.

Weight watchers

Are you looking for a balance, but either space is at a premium or portability is desirable. Lux Scientific may have the answer. The company is offering Pocket Pro **pocket-sized balances**, which weigh in g, oz, dwt and



Lux's lightweight balances.

ozt, and are available in 80-g, 150-g and 250-g capacities *(Reader Service No. 108).* These battery-operated digital balances are graduated n 0.1-g increments and have a precision of ±0.1 g, says Lux. Each Pocket Pro balance is supplied with a calibration weight and carrying case.

All-round access and visibility are the key design features of the RC Series of **analytical balances** from Sartorius *(Reader Service No. 109).* Access to the weighing chamber is achieved by turning the movable draft shield and adjusting the width of the opening to suit. With a single keystroke, the door will close and the opening position will be held in the instrument's memory for future applications. The door can be opened to an extra-wide angle of 170 ° for robotic weighing applications. The RC Series includes balances with weighing ranges from 0–60 g and from 0–250 g, and a readability range of 0.01–0.1 mg. Response times are less than 10 seconds, says Sartorius. An automatic calibration function compensates for any drift that may occur as a result of adverse ambient conditions. The RC Series can be connected to computers for remote control operation. □

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Manuscripts are selected for publication according to editorial assessment of their suitability and reports from independent referees. They can be sent to London or Washington and should be addressed to the Editor. Manuscripts may be dealt with in either office, depending on the subject matter, and will where necessary be sent between offices by overnight courier. All manuscripts are acknowledged on receipt but fewer than half are sent for review. Those that are not reviewed are returned as rapidly as possible so that they may be submitted elsewhere without delay. Contributors may suggest reviewers; limited requests for the exclusion of specific reviewers are usually heeded. Manuscripts are usually sent to two or three reviewers, who are chosen for their expertise rather than their geographical location. Manuscripts accepted for publication are typeset from the London office.

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Categories of paper

Review articles survey recent developments in a field. Most are commissioned, but suggestions are welcome in the form of a one-page synopsis addressed to the Reviews Coordinator. Length is negotiable in advance.

Articles are research reports whose conclusions are of general interest and which are sufficiently rounded to be a substantial advance in understanding. They should not have more than 3,000 words of text (including figure legends) or more than six display items (figures and tables) and should not occupy more than five pages of *Nature*.

Articles start with a heading of 50–80 words written to advertise their content in general terms, to which editors will pay particular attention. The heading does not usually contain numbers, abbreviations or measurements. The introduction to the study is contained in the first two or three paragraphs of the article, which also briefly summarize its results and implications. Articles have fewer than 50 references and may contain a few subheadings of two or three words.

Letters are short reports of outstanding novel findings whose implications are general and important enough to be of interest to those outside the field. Letters should have 1,000 or fewer words of text and four or fewer display items. The first paragraph describes, in not more than 150 words and without the use of abbreviations, the background, rationale and chief conclusions of the study for the particular benefit of non-specialist readers. Letters do not have subheadings and contain fewer than 30 references.

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Scientific Correspondence is for discussion of topical scientific matters, including those published in *Nature*, and for miscellaneous contributions. Priority is given to letters of fewer than 500 words and 5 references.

Preparation of manuscripts

All manuscripts should be typed, double-spaced, on one side of the paper only. An original and three copies are required, each accompanied by artwork. If photographs are included, four sets of originals are required; for line drawings, one set of originals and three good-quality photocopies are acceptable. Reference lists, figure legends and tables should all be on separate sheets, all of which should be double-spaced and numbered. Relevant manuscripts in press or submitted for publication elsewhere should be included with each copy of a submitted manuscript, and clearly marked as such. Revised and resubmitted manuscripts should also be clearly marked as such and labelled with their manuscript numbers.

Titles say what the paper is about with the minimum of technical terminology and in fewer than 80 characters in the case of Articles and Letters. Active verbs, numerical values, abbreviations and punctuation are to be avoided. Titles should contain one or two key words for indexing purposes.

Artwork should be marked individually and clearly with the author's name and, when known, the manuscript number. Ideally, no figure should be larger than 28 by 22 cm. Figures with several parts are to be avoided and are permitted only if the parts are closely related, either experimentally or logically. Unlettered originals of photographs should be provided. Original artwork is returned when a manuscript cannot be published.

Protein/nucleotide sequences should ideally be in the three-letter and not the single-letter code for amino acids. One column width of *Nature* can accommodate 20 amino acids or 60 base pairs. Numbering of sequences should be in the left-hand margin only, with a single space between rows.

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References are numbered sequentially as they appear in the text, followed by those in tables and finally by those in figure legends. Only papers published or in the press are numbered and included in the reference list. All other forms of reference, including unrefereed abstracts, should be cited in the text as a personal communication, manuscript submitted or in preparation. Text is not included in reference lists. References are abbreviated according to the *World List of Scientific Periodicals* (Butterworths, London, 1963–65). The first and last page numbers are included; reference to books should include publisher, place and date.

Abbreviations, symbols, units and greek letters should be identified the first time they are used. Acronyms should be avoided whenever possible and, if used, defined. Footnotes are not used except for changed addresses.

Acknowledgements are brief; grant and contribution numbers are not allowed.

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DYSON PERRINS LABORATORY, UNIVERSITY OF OXFORD

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Applications are invited from biochemists and organic chemists to work in the research group of Dr. S. L. Flitsch on an interdisciplinary project involving the Chemoenzymatic Synthesis of Oligosaccharides and Glycoproteins (J. Chem. Soc., Chem. Commun. 1991 pages 380-382 and 382-384). The organic chemist should have some experience in synthesis (not necessarily carboyhydrate chemistry) and an interest in biological chemistry. The biochemist should be interested in the purification of soluble and membrane bound enzymes. Further information about the posts can be obtained from Dr. S. L. Flitsch (Tel. 0865-275697).

Applicants should write to **The Administrator, Mr G. Hurst, The Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY, GB** as soon as possible enclosing a full *curriculum vitae*, and arrange for two referees to write directly on their behalf. The starting date is October 1991 or as soon as possible thereafter. The position is tenable for 1 year at this stage and renewable to up to three years. The stipend will be on the University RA1A research scale £11,399-£13,495 p.a. according to age and experience plus USS.

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Cancer Research Campaign

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A Postdoctoral Fellow to work on embryos and development tumours;

A Research Assistant to work on the molecular cloning of a new proteoglycan;

and a Technician to assist in cell culture, immunochemistry and molecular biology.

Experience in cell or molecular biology would be helpful.

Applications, including a CV and the names and addresses of two academic referees, and informal enquiries should be made to: Dr. M. F. Pera, Room E148, Department of Zoology, South Parks Road, Oxford, OX1 3PS.

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Required to join a research programme on the isolation and analysis of mammalian genes involved in DNA repair, replication of damaged templates and cell cycle responses to damage. Expertise in DNA repair is not essential but the successful candidate will be experienced in the isolation, cloning, organization and regulation of eukaryote genes.

The post is available immediately and will extend in the first instance to 31st December 1991. Salary will depend on age and experience but is expected to start within the Cambridge University Research Assistant – Research Associate scales (£12,792 at age 26 to £16,755 at age 32). In line with CRC policy there will also be a salary enhancement equivalent to two points on the salary scale. Applications (quoting reference RTJ) including CV and giving the names of two referees, should be sent to the Secretary, Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ. Informal information can be obtained from Dr. R. T. Johnson (0223 336672).

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SENIOR SCIENTIST

A tumour biologist with experience in radiobiology, flow cytometry, growth factors or vascular biology is required to lead a small research group (4-6 graduates) in a multidisciplinary environment. The person appointed will be expected to direct their own projects aimed at improvement of cancer therapy.

Salary in the range of £26,697 – £30,046 (including London Weighting and a non-superannuable CRC enhancement equivalent to two increments on the salary scale, subject to annual review) will be commensurate with age and experience.

For further details please write to Professor J Denekamp, CRC Gray Laboratory, P.O. Box 100, Mount Vernon Hospital, Northwood, Middlesex HA6 2JR, including a full CV, a brief outline of your research proposal and names and addresses of three academic referees. (6212)A

Fighting cancer on all fronts.

POST DOCTORAL RESEARCH ASSISTANTSHIPS

The School of Materials, University of Sheffield and The Materials Science Centre, University of Manchester/UMIST are two of seven partners in a BRITE/EURAM Project on Microstructural Modelling of Industrial Thermomechanical Processing of Aluminium Alloys.

This three year project commences 1st July 1991 and requires a Research Assistant for studies of plane strain compression and rolling deformation behaviour at Sheffield, and a Research Assistant for detailed microstructural and microtexture studies at Manchester.

Applicants should have a Ph.D in Materials Science or a related subject. Salary within the range of £12,792 — £14,038 depending on age and experience.

Applications, including CV, should be sent to either, or both:

Professor C M Sellars, University of Sheffield, School of Materials, Sir Robert Hadfield Building, Mappin Street, Sheffield S1 3JD. Professor F J Humphreys, The Materials Science Centre, Grosvenor Street, Manchester M1 7HS.

Closing date: 14 June 1991. Informal enquiries to Professor C M Sellars (0742 768555, ext. 5511) (Fax: 0742 754325) or to Professor F J Humphreys (061 2003554) (Fax: 061 2003586). Ref. MAP391/G. (6194)A

UNIVERSITY OF KENT AT CANTERBURY

Pfizer Chair of Medical Statistics

The Pfizer Chair of Medical Statistics, in the Institute of Mathematics and Statistics, has been created as a result of general financial support from Pfizer Limited. Applications are invited for the Chair from persons who have an excellent record of research in Medical Statistics and preferably also proven ability in teaching and administration. As a member of an already strong group of statisticians, the successful candidate is expected to contribute substantially to the widening of our research activities, course development and links with industries, especially in the direction of medical statistics of relevance to medicine and the pharmaceutical industry. We would particularly welcome applicants with expertise in Clinical Trials, Statistical Modelling in Medicine and Biology, Statistical Software, Statistical Issues of Medicine Regulations and New Technology in Medicine. Other areas are not excluded.

Salary within the Professional range (minimum £27,013 per annum). The appointment will be for a fixed term of five years. Further particulars (including details of the application procedure) are available from the Personnel Office, The Registry, The University, Canterbury, CT2 7NZ (telephone (0227) 764000 extension 3915) quoting reference number A91/53. Informal enquiries may be made to Professor Howell Tong at the same address (telephone extension 3658).

Closing date for applications: 30th June, 1991.

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DEPARTMENT OF ANATOMY

Lecturer in Cell Biology

(Limited Tenure)

The Department of Anatomy wishes to appoint a Lecturer with teaching and research experience in the field of cell biology.

Teaching. The Department is responsible for courses in cell biology (including histology) for medical, dental, science and physiotherapy students, with advanced courses in the science stream.

Research. The appointee is expected to actively contribute to the existing research of the Department, either by establishing a laboratory, or by joining one of the research groups in the Department. Current research in the Department is in 3 main fields: the renal glomerulus and juxtaglomerular apparatus; the primate sensorimotor system; and developmental biology of the nervous and endocrine systems.

In addition to the specific research laboratories, the general facilities of the Department include well-equipped histology, electron microscopy (including a CM12 STEM), cell culture, molecular biology, stereology, image-analysis, and neurobiology laboratories. A primate breeding colony is also closely linked to the Department.

The appointee will have laboratory space, and use of the above facilities. In 1992 limited Departmental funds will be available for starting a research program. Faculty and University-wide funds are available each year for new members of staff. After 1992 the major funding of the appointee's research must be obtained from other sources such as the NH&MRC and ARC.

Appointment. The initial appointment is for 3 years, but a continuing appointment is possible thereafter. The position is available from November 1991, and should be taken up no later than 1 January 1992, as undergraduate teaching starts a month later, and importantly, applications for research support from the University and other granting bodies must be submitted late in 1991 or early in 1992.

Salary: $A33,163 – $A43,096 p.a. Medical and Dental graduates receive additional salary loadings.

Further information and a position description: Professor Ian Darian-Smith (613) 344 5804; facsimile (613) 347 5219.

Applications close: 28 June 1991. Reference number: B/516/118.

Applications should be sent, in duplicate, quoting three referees (including facsimile numbers) to the Director, Personnel Services, The University of Melbourne, Parkville, Victoria, 3052, Australia.

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arm48122

POSTDOCTORAL POSITIONS IN NEUROBIOLOGY

Studies of nicotinic acetylcholine receptors of muscles and nerves and the autoimmune response to nicotinic receptors of muscle require candidates with MD or PhD degrees experienced with either **molecular genetics** (for cDNA cloning, mutagenesis, and expression), or experienced with **protein biochemistry and monoclonal antibodies** (for receptor purification and characterization, and monoclonal antibody production, and studies of subunit synthesis and assembly), experienced with **electrophysiology** (for studies of receptors expressed from cDNAs in *Xenopus* oocytes and transformed cells), or experienced with **histology** (for immunohistological localization of subunit proteins and in situ hybridization localization of subunit mRNAs). For the right candidates, there is opportunity to work in several of these areas in a highly productive lab.

Send your curriculum vitae and names, address, and telephone numbers of three references to: **Dr. Jon Lindstrom, Institute of Neurological Sciences, University of Pennsyvania School of Medicine, 215 Medical Education Bldg., Philadelphia, PA 19104-6074. Fax (215) 573-2015.**

(NW6676)A

UNIVERSITY OF YORK CANCER RESEARCH UNIT

DEPARTMENT OF BIOLOGY

(Research Positions)

One in four of the UK population will die from cancer if present trends continue. The search for the causes of cancer is a major international research endeavour with which our Unit is heavily involved. We need two lively and independent individuals to join our active research programmes.

Position 1 – Post-doctoral or Graduate Research Fellow

This position, funded by The Imperial Cancer Research Fund, is to investigate reasons for susceptibility to the commonest form of cancer, skin cancer. Studies will involve examining DNA repair in skin biopsy tissue from individuals whose skin has been irradiated with UV. Applicants should either be at the post-doctoral level or be Biological graduates with, or expecting to gain, a 2i Honours degree. Graduates would be expected to register for a PhD (Reference no. 7/6349).

Position 2 – Research Assistant

This position, funded by the European Community's Environmental Programme, is concerned with studying urban and rural differences in cancer incidence by studying DNA damage in organs such as the lung, bladder, etc. In addition, surrogate dose monitors for exposure will be sought, such as adduction with lymphocyte DNA. Applicants should have, or expect to gain a 2i Honours degree in a biological science. The successful applicant would be expected to register for a PhD (Reference no. 7/6350).

Salary scale Position 1: £11,399-£15,444 per annum within the scales for research staff, depending upon age and experience.

Position 2: £11,399-£13,495 per annum within range 1B of the scales for research staff.

Three copies of applications with full CV and the names of two referees, should be sent by 31 May 1991 to the Personnel Office, University of York, Heslington, York YO1 5DD: please quote appropriate reference number.

Informal enquiries may be made to Dr Colin Garner on (0904) 432900.

(6190)A

UNIVERSITY OF NEWCASTLE UPON TYNE
Department of Biochemistry and Genetics

POSTDOCTORAL RESEARCH ASSOCIATE IN ENZYMOLOGY

Applications from biochemists or chemists are invited for a three-year appointment within a programme supported by the SERC. This post will be involved in the characterisation of the active site and catalytic mechanism of penicillin acylase to underpin protein engineering of the enzyme. The project will provide training in a range of techniques in protein-biochemistry with relevance to biotechnology.

Salary will be up to £13,495 per annum on the Grade 1A scale: (£11,399 — £18,165), according to qualifications and experience, and the appointment will commence as soon as possible.

Enquiries and applications, including a curriculum vitae and the names of three referees to Dr R Virden, Department of Biochemistry and Genetics, University of Newcastle upon Tyne, NE2 4HH (Tel: 091 222 7432 or 091 222 6000, extension 8126) to arrive not later than 31st May, 1991.

(6157)A

STAFF SCIENTIST MOLECULAR/CELLULAR BIOLOGY

The Biology Division of the Oak Ridge National Laboratory is seeking an investigator to establish a research program involving targeted mutagenesis in transgenic mice. A PhD (molecular biology, biochemistry, or cell biology) with postdoctoral experience and demonstrated potential to attract extramural support is required. Core funding will be provided at a level commensurate with the candidate's qualifications. Prior experience with embryonic stem cells and/or transgenic mice is preferred but not essential. The Biology Division of 30 senior staff members is engaged in diverse research related to health effects of energy production and utilization for the Office of Health and Environmental Research within the Department of Energy. The newly-created staff position reflects increased emphasis in functional genomic mapping and gene regulation *in vivo* and complements established programs in molecular biology that include insertional mutagenesis in mice.

Qualified applicant should forward resume, publication list, and names of three references to Dr. Fred C. Hartman, Director, Biology Division, Dept. NA-0418, P.O. Box 2009, Oak Ridge National Laboratory, Oak Ridge, TN 37831-8077. ORNL which is managed by Martin Marietta Energy Systems, Inc. for the USDOE, is an equal opportunity employer.

ornl

(NW6622)A

immunex®

SCIENTIST IMMUNOLOGY

Immunex Corporation, a Seattle pharmaceutical company focused on the research, development, marketing and manufacturing of immunological therapeutic products is currently recruiting for a Scientist to join our Immunology group.

The successful candidate will have experience in rodent models in inflammation or autoimmunity. This individual will join a multi-disciplined group of scientists studying cytokines and their antagonists in vivo.

Scientists with a Ph.D in Immunology, or related discipline with relevant post doctoral/industry experience are encouraged to apply. Good communication skills and the ability to work effectively in a collaborative environment are essential.

Immunex Corporation offers a fast paced, stimulating environment, competitive salaries and an excellent benefits package. For consideration, please submit C.V., names of three references, and a list of publications to:

Immunex Corporation, Attn: Human Resources Dept, 51 University Street, Seattle, WA 98101. Fax: (206) 587-0606.

Equal Opportunity Employer. (NW6699)A

PROTEIN X-RAY CRYSTALLOGRAPHER

National Institute of Allergy and Infectious Diseases

National Institutes of Health

A protein crystallography laboratory is being established with intramural AIDS funding to conduct an original research program and to interact with other scientists within the NIH intramural program. Applicants for director of the crystallography lab are being sought. Ph.D. or M.D. applicants should have a record of achievement in solving protein structures by X-ray crystallography. Salary and research resources will be commensurate with credentials and research experience.

Applicants should send curriculum vitae and names and addresses of at least three references to:

John E. Coligan, Ph.D.
Chairman, Search Committee
BRB, NIAID, NIH
Building 4, Room 413
9000 Rockville Pike
Bethesda, MD 20892
Telephone: 301-496-8247
Facsimile: 301-402-0284

NIH is an Equal Opportunity Employer

(NW6668)A

The Research Centre for Arthritis and Autoimmunity announces positions for

Five Senior Staff Scientists

Located at The Wellesley Hospital and affiliated with The Department of Immunology, University of Toronto

The Centre has been formed to study the fundamental components of the immune system and thereby contribute to better understanding of Autoimmune Disease. We are currently seeking highly motivated individuals with research interests in:

- BIOCHEMISTRY: Assembly, processing, structure/function relationship of proteins such as the MHC, antigen receptors, signal transduction molecules, hormone receptors, growth factors/receptors.
- MOLECULAR BIOLOGY: Genetic mechanisms which initiate and regulate the immune response, repertoire formation, lymphocyte growth and differentiation, and cellular interactions.
- CELLULAR BIOLOGY: Cellular regulation of immunity, models of autoimmune disease, mechanisms of cell selection and expansion.

In the selection of candidates consideration will be given to: a demonstrated track record of producing high quality science; the ability to develop and maintain independent, externally funded research programs; a willingness to interact with other members of the Department, a citywide group of 40 scientists, and with the clinical research staff of The Wellesley Hospital in Endocrinology, Rheumatology, Inflammation Research; and evidence of teaching ability. A PhD or eqivalent degree, as well as several years postdoctoral experience, are required. Academic rank, from assistant to full professor, the the Department of Immunology will be determined based on qualification and experience. A competitive package including salary, benefits, space, and start-up funds will be provided starting July 1, 1991. Interested individuals should send, by July 1st, 1991, their curriculum vitae and a few representative publications to: **Dr Christopher J Paige, Director, The Wellesley Hospital Research Institute, 160 Wellesley Street East, Toronto, Ontario M4Y 1J3.**

The Wellesley Hospital is a fully affiliated University of Toronto Teaching Hospital.

(NW6690)A

KINGSTON POLYTECHNIC

▪ FACULTY OF SCIENCE

SCHOOL OF GEOLOGICAL SCIENCES

Lecturer/Senior Lecturer (2 Posts)

in Applied and/or Engineering Geology (1) and Geophysics (2)

Up to £24,090 pa inc.

We are looking for an applied/engineering geologist and a geophysicist to join an enthusiastic group of staff in a well-established Geology department. You will be involved in either the teaching of applied and engineering geology or the principles and application of geophysics to students undertaking degrees in Geology, Earth Science and Civil Engineering. The teaching duties may also include courses in mineral exploration, engineering geology and geophysics to students on MSc programmes organised by the School.

Preference may be given to those with industrial experience, for example, in the oil industry or in mineral exploration, mining or civil engineering. The School has a strong commitment to research and encourages links with industry and you will, therefore, be expected to undertake and develop research and/or consultancy activities within the School. You should have an honours degree in a relevant subject and a postgraduate qualification or professional experience.

The appointments would be from 1st September 1991 and may be permanent or for a two year fixed term depending on experience.

Please quote ref: JS 938/N (post 1) or JS 939/N (post 2).

For further details and an application form please contact the Personnel Department, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames, Surrey KT1 2EE. Telephone 081-549 1366 extension 2153, quoting the appropriate reference number. Closing date: 7th June 1991.

We are an Equal Opportunities Employer (6224)A

The University of Calgary
NEUROPHYSIOLOGIST

The University of Calgary Department of Medical Physiology invites applications for a neurophysiologist working in the area of control of breathing. Qualifications include a PhD and two years' postdoctoral training, proven expertise in the area of fundamental aspects of neurophysiology and neurobiology with application to respiratory motor control of breathing, and respiratory rythmogenesis. Expertise in *in vitro* techniques, and expertise with neural cell culture are considered important. The selected candidate must compete successfully for salary and establishment support through application to the Alberta Heritage for Medical Research and/or the Medical Research Council of Canada, will have 75% of time protected for research, and will be associated with the Respiratory Research Group of the Faculty of Medicine.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. The University of Calgary has an Employment Equity Programme and encourages applications from all qualified candidates, including women, aboriginal people, visible minorities and people with disabilities.

Please submit a curriculum vitae, statement of research interests, and the names of three reference by June 15 1991, to: **J. E. Remmers, M.D., Chair, Respiratory Research Group, The University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta, Canada T2N 4N1.** (NW6696)

UNIVERSITY OF
essex

Department of Biology

SENIOR RESEARCH OFFICER
Microbial oxidation of methane

Applications from microbiologists are invited for the post of Research Officer to investigate the oxidation of methane by micro-organisms in the cover soil above landfill sites. The work, funded by the Department of the Environment, aims to optimise methane oxidation to reduce the environmental impact of methane emission.

A postdoctoral Senior Research Officer (Grade IA £11,399-£18,165, under review) will be preferred, but well qualified post-graduates (Grade IB £11,399-£13,495 under review) will also be considered and if appointed may register for a higher degree. The appointment is for three years. Experience of interfacing equipment with dataloggers or microcomputers will be an advantage, and a driving licence is essential for field work.

Prospective applicants may discuss the project informally with Dr D B Nedwell (0206 872211).

Applications (3 copies), including a curriculum vitae and the names and addresses of two academic referees, should reach The Registrar & Secretary (R/175/N), University of Essex, Wivenhoe Park, Colchester CO4 3SQ by not later than 24th May 1991. Further particulars may be obtained by telephoning Colchester (0206) 872462 (24 hours). (6184)A

IMMUNOLOGY FACULTY POSITION

The Department of Veterinary Microbiology and Parasitology invites applications for an Assistant/Associate Professor position. The successful candidate will participate in teaching veterinary and graduate immunology, in the guidance of graduate students, and will develop a strong research program. Applicants must have a PhD or DVM/PhD degree in immunology. Post-doctoral experience with significant research accomplishments in contemporary aspects of immunology and immune responses to infectious or parasitic agents would be beneficial.

Send a letter of interest with *curriculum vitae* and three references to: **Dr T R Klei, Search Committee Chair, Department of Veterinary Microbiology and Parasitology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803, U.S.A.** Application deadline is June 15, 1991 or until a suitable applicant is identified.

LSU is an affirmative action/equal opportunity employer. Women and minorities are encouraged to apply. (NW6700)A

VIROLOGIST
University of Maryland at Baltimore

A tenure-track molecular virologist position is available in the Department of Microbiology and Immunology at the University of Maryland School of Medicine in Baltimore. The position can be filled at the Assistant or Associate Professor level, commensurate with the experience of the successful candidate. Applications are encouraged from individuals with molecular expertise in any area of animal virology. The successful applicant will be expected to establish a vigorous independent research program, participate both in graduate and medical school teaching, and interact with other faculty members with virological interests. The position carries attractive start-up funds as well as a competitive salary and benefit package.

Interested parties should send a recent CV, plus relevant reprints, the names of three references, and a short statement of their proposed research program to: George K Lewis, PhD, Virology Search Committee, Department of Microbiology and Immunology, University of Maryland, 655 W. Baltimore St., Baltimore, MD 21201.

The University of Maryland at Baltimore is an equal opportunity employer. M/F/H/V (NW6701)A

THE AUSTRALIAN NATIONAL UNIVERSITY

THE JOHN CURTIN SCHOOL OF MEDICAL RESEARCH

DIVISION OF CLINICAL SCIENCE
POSTDOCTORAL FELLOW (LYMPHOCYTE BIOLOGY)

Applications are invited from PhD graduates in either immunology, molecular biology or virology, to work in collaboration with Dr H O'Neill on a research project supported by the National Health and Medical Research Council "Lympho-proliferation induced by murine retroviruses". Appointment will be for two years initially, with the possibility of extension, following review, for a third year. Enquiries to Dr H O'Neill, Experimental Haematology Group, (tel. (61 6) 2494720; Fax (61 6) 2490413).
Closing date: 31 May 1991. Ref: JC 23.4.1.

DIVISION OF CELL BIOLOGY
POSTDOCTORAL FELLOW,
VIRAL IMMUNOLOGY BIOLOGY

A position at Postdoctoral level is available immediately. The successful applicant will work in collaboration with Drs P Waring and A Mullbacher on aspects of viral infections and the ensuing cell death mediated by immune mechanisms. Experience in biochemistry, molecular biology, immunology and/or virology is desirable. Appointment will be for two years initially, with the possible extension, after review, into a third year terminating no later than December 1993.
Closing date: 31 May 1991. Ref: JC 23.4.2.

DIVISION OF CELL BIOLOGY
POSTDOCTORAL FELLOW
(CELLULAR IMMUNOLOGIST)

An externally funded position at Postdoctoral level is available immediately in the Division of Cell Biology. The position is available for an individual with a PhD and experience in cellular immunology. The successful applicant will work with Drs Mullbacher and Waring on aspects of immunomodulation with special reference to immunosuppression by certain drugs and their effects on transplantation in the murine system and on cell of human origin. Appointment will be for two years initially, with extension, following review, possibly into a third year, but not later than 31.12.93. Enquiries to Dr A Mullbacher, (tel. (61 6) 2494392; Fax (61 6) 2492595).
Closing date: 31 May 1991. Ref: JC 23.4.3.

SALARY: A$28,792 — A$32,762 pa; APPLICATIONS should be submitted in duplicate to the Registrar, The Australian National University, GPO Box 4, Canberra ACT 2601, Australia, quoting reference number and including curriculum vitae, list of publications and names of at least three referees. The University reserves the right not to make an appointment or to appoint by invitation at any time. Further information is available from the Registrar, or from the Appointments Officer, Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF. (W8467)A

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER

Synthetic Organic Chemist

Corvas, International, Inc., a biopharmaceutical company with research centers in La Jolla, California and Ghent, Belgium is expanding to address exciting product opportunities in the thrombosis and vascular disease field. We are seeking an outstanding synthetic organic chemist to join our La Jolla laboratory. Qualified candidates will have a PhD, post-doctoral, and prior industrial experience.

You will design and synthesize enzyme inhibitors and receptor ligands and will focus on peptidyl mimetic chemistry, constrained amino acid analogs, and non-peptidyl drug candidates. A strong background in synthetic techniques and spectroscopic analysis is necessary. In your work you will collaborate with a strong molecular modeling group in drug design.

Corvas offers attractive salaries and benefits, an excellent opportunity for equity participation and a highly interactive scientific environemnt. For consideration please send a letter of interest and a CV to:

Human Resources Director,
Corvas International, Inc.,
3030 Science Park Road,
San Diego, California 92121

CORVAS, INTERNATIONAL, INC.

(NW6667)A

Advertisement for Faculty Positions in Molecular Regulation of Cellular Signalling

The Department of Pharmacology at the Duke University Medical Center is increasing its faculty as a part of a major expansion in the basic sciences. The department wishes to enhance its focus on molecular regulation of cellular signalling. Applicants are being sought who have a research background utilizing modern molecular biological and/or genetic technology and are interested in working in a collegial and interactive environment. Most appointments will be at the level of assistant professor. Please send curriculum vitae, list of three references and a brief description of proposed research to: **Dr. Anthony R. Means, Chairman, Department of Pharmacology, Box 3813, Duke University Medical Center, Durham NC 27710.** Duke University is an Equal Opportunity/Affirmative Action Employer. (NW6679)A

NSERC
Women's Faculty Award

The **Department of Geology of McMaster University** is looking for a candidate for an NSERC Women's Faculty Award for the Academic year 1992-93. The Department of Geology at present consists of eleven full-time, five part-time and two emeritus professors, plus six associate members in other departments. We have recognized strengths in the fields of geochemistry, both elemental and isotopic; clastic sedimentology; and biogeology. We have an ongoing commitment to expand our instruction and research in the field of environmental geology and geochemistry.

Emphasis would be put on evidence of proven record of accomplishment. Candidates should have completed a PhD degree or expect to have completed it by the time that they would take up the position in the summer of 1992.

Applicants should provide the names of two referees who can attest to their abilities in research and teaching.

Preference will be given to Canadian citizens or Landed Immigrants.

Interested applicants should write to Dr A P Dickin, Chair, WFA Search Committee, Department of Geology, McMaster University, Hamilton, Ontario L8S 4M1.

Deadline for receipt of applications is September 1st, 1991.

(NW6653)A

Department of Experimental Medicine
COLLLEGE DE FRANCE – PARIS – FRANCE
POSTDOCTORAL RESEARCH POSITION

Available for 1 or 2 years to study the action of angiotensin II in transmembrane signaling. The approach involves combined application of recombinant DNA techniques and biochemical analysis of second messengers, including unicellular recording of Ca transients. Expertise in these fields will be an advantage.

French citizen applications cannot be considered.

Salary range: FF 100.000 (according to age and experience).

Please send CV + name of two references to:
Doctor Eric Clauser — INSERM U36
(Pr P. Corvol), Collège de France - 3,
rue d'Ulm - 75005 Paris, France.

(W8463)A

UNIVERSITY OF OXFORD

Institute of Molecular Medicine
Paediatric Molecular Genetics
John Radcliffe Hospital

POSTDOCTORAL RESEARCH ASSISTANT
(Grade RS1A) £11,399 to £18,165 per annum

GRADUATE RESEARCH ASSISTANT
(Grade RS1B) £11,399 to £15,444 per annum
(Both the above positions are available for 3 years, in the first instance)

POSTDOCTORAL RESEARCH ASSISTANT
(Grade RS1A) £11,399 to £18,165 per annum

GRADUATE RESEARCH ASSISTANT
(Grade RS1B) £11,399 to £15,444 per annum
(Both the above positions are available for 18 months, in the first instance)

Applications are invited for the above four posts on research investigating the function of the cystic fibrosis (CF) gene. Research projects in the programme include regulation of expression of the CF gene in the specialized epithelial cells that express the basic defect; developmental expression of the CF gene; and definition of mutations and their biological effects. All the posts are funded by the Cystic Fibrosis Research Trust and are available from July 1st, 1991. Experience in molecular and/or cell biological techniques would be an advantage.

Further details are available from Dr Ann Harris (0865 221070).

POSTDOCTORAL RESEARCH ASSISTANT
(Grade RS1A) £11,399 to £18,165 per annum

Applications are invited for a postdocioral research assistant to work on a research project investigating the nature of mutations in patients with metachromatic leukodystrophy or Gaucher's Disease. This is a joint project with the Division of Medical and Molecular Genetics at Guy's Hospital, London, and the Paediatric Molecular Genetics Group in the Institute of Molecular Medicine at Oxford. The successful applicant will be based in Oxford. The position funded by the Medical Research Council, is for 3 years in the first instance, and is available from July 1st, 1991.

Further details are available from Dr Ann Harris (0865 221070) or Dr Tony Fensom (071 955 4646).

Applications in writing for all posts together with a full curriculum vitae and the names and addresses of two referees, should be sent to the Departmental Administrator, Department of Paediatrics, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU. Closing date for applications: May 24th, 1991. (6223)A

The University is an Equal Opportunity Employer

UNIVERSITY OF SURREY
School of Biological Sciences

CHAIR IN BIOLOGICAL SCIENCES

Nutrition at the Cellular and Molecular Level

Applications are invited for a Chair in the School of Biological Sciences. The position is one of a series of strategic appointments associated with the development of the newly created School, which is a major initiative in the biological and health sciences in the University of Surrey. Scientists with an exceptional research record, particularly in the application of cellular and molecular biology to fundamental biochemical aspects of nutrition, are encouraged to apply. The School has well rounded research strengths in the interactions between dietary components and human health, and the person appointed will be expected to provide leadership for this group and development of the molecular and cellular science capability to advance nutritional research. The commencing salary is expected to be not less than £33,000 per annum.

Further particulars may be obtained from the Personnel Office (CVJ), University of Surrey, Guildford, Surrey GU2 5XH (tel: 0483 509279). Applications in the form of a curriculum vitae (3 copies) including the names and addresses of three referees should be sent to the above address by 31 May 1991 quoting reference number 017. (6198)A

UNIVERSITY OF CAMBRIDGE
Department of Medicine

RESEARCH ASSISTANT

A position is available immediately for a research assistant with an interest in human genetics to study disease loci that are inherited on the human X-chromosome. Previous experience in the techniques of molecular biology and/or mammalian cell culture will be an advantage. The position is funded for three years on an age related scale with a salary of up to £13,495 with the possibility of registering for a PhD.

Further information can be obtained from Dr Sue Kenwrick at the Department of Medicine, Level 5, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QQ. Telephone 0223 336859. Applications should be sent to the above address quoting ref: MKC3977 and include a CV and the names and addresses of three referees.

The University follows an equal opportunities policy. Addenbrooke's Hospital is a no-smoking area. (6196)A

THE ROWETT RESEARCH INSTITUTE

BONE CELL BIOLOGIST/ BIOCHEMIST

Applications are invited for a post-doctoral appointment in the Bone Growth and Metabolism Group to work on the control of bone cell differentiation with Drs B. M. Thomson and N. Loveridge. The work will involve the characterisation of growth-factor responsive populations of skeletogenic precursor cells, using cell and molecular biology, immunocytochemistry and image processing techniques.

The Rowett is an independent Research Institute, linked to the AFRC, with a strong emphasis on the application of basic molecular and cellular approaches to animal and human biology and the nutritional sciences. Situated on the outskirts of Aberdeen, it has extensive links with the University and with other research/academic centres in the area. Aberdeen is a thriving city, with a strong cultural base and excellent facilities for outdoor recreational activities.

The appointment will be made on a fixed term, 3 year basis at the HSO level (£11,586-£16,176), with initial placing according to qualifications and experience. The Institute is an Equal Opportunities Employer and a non-contributory pension scheme is in operation.

Applications should be forwarded to the personnel officer, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, Scotland within 1 month of the appearance of this advertisement. Candidates are invited to discuss the post informally with Dr B. M. Thomson (Tel. 0224-712751 ext. 243). (6179)A

INSTITUTE OF CHILD HEALTH
University of London
DIVISION OF CELL AND MOLECULAR BIOLOGY

Post Doctoral Research Fellow

To work on the signal transduction pathways regulating human B lymphocyte growth and differentiation. The position is in the Cellular Immunology Unit headed by Dr. R. Callard.

There will be opportunities to develop cellular, biochemical and molecular techniques to investigate the role of cytokines and cell surface receptors in the regulation of human B cell responses.

The post is available immediately and is for 3 years.

Salary on RA IA scale according to age and experience.

Applications with CV to Dr. R. Callard, Division of Cell and Molecular Biology, Institute of Child Health, 30 Guilford Street, London, WC1N 1EH, U.K. Telephone: 071-2429789. Telefax: 071-8314366. (6204)A

IRRI

INTERNATIONAL RICE RESEARCH INSTITUTE

Office of the Director General

POSITION ANNOUNCEMENT FOR

SOIL MICROBIOLOGIST

The International Rice Research Institute (IRRI), a member of the Consultative Group on International Agricultural Research (CGIAR), is an international, nonprofit, donor-funded, agricultural research institute that conducts research and training at its headquarters in the Philippines and collaborates with national agricultural systems worldwide. IRRI's goal is to develop more productive and sustainable rice technology and to strengthen national research capability.

IRRI seeks a soil microbiologist for assingment to its headquarters at Los Baños, Laguna, Philippines, to work within teams of agronomists, soil scientists, plant physiologists, breeders, and economists to generate knowledge and develop technologies for rice ecosystems as part of the IRRI Strategy and Five-year Workplan.

Position description

The soil microbiologist is assigned to the Soil Microbiology Division. Under IRRI's matrix management system he/she will contribute to projects of the irrigated Rice and Rainfed Rice Programs of IRRI.

- As project member or project coordinator he/she will work in an interdisciplinary and international approach to:
  1. Generate knowledge on microbial/biological processes in rice soils and at the soil-rice root interface to
  – improve nutrient cycling and use by rice.
  – improve rice tolerance to microbiologically governed stresses, and
  – assess environmental impacts of rice production systems.
  2. Contribute to the development of technologies to reduce dependence on purchased nutrients, with emphasis on nitrogen cycling and biological nitrogen fixation.
  3. Contribute to the development of environmentally sound rice cultivation.
- As a member of the Soil Microbiology Division he/she will:
  1. Supervise the facilities devoted to Soil Microbiology and the preservation of the biofertilizer germplasm collection.
  2. Ensure constant updating of microbiological and anlytical methods used in the laboratories.
- As a member of an international research organization he/she will:
  1. Train scientists from national agricultural research systems (NARS) in soil microbiology and contribute to the general training programs of IRRI.
  2. Develop collaborative research with advanced institutions and NARS.

Salary and benefits are competitive with those of similar international institutions. The position offers an opportunity to interact with an international and highly motivated group of scientists, and to positively influence rice-related research in Asia and the world.

Qualification

Applicants should have a PhD or equivalent in soil sciences or biology with specialization in soil microbiology and microbial ecology, and proven experience in science leadership and research management. A good knowledge of methods for field studies in microbial ecology is needed. Skills in statistics, methods of analysis in ecology, and computer use are desirable. Experience in field research and/or rice research is preferred. English fluency is essential. Further language abilities are desirable. Ability to interact with other research disciplines and people from various nationalities is essential.

Application

Please submit curriculum vitae, date of availability, and the names of three referees to:

Dr. Klaus Lampe, Director General
PO Box 933, 1099 Manila, Philippines
Tel. no. (63·2) 818-1926 · FAX: (63·2) 817-8470
Telex: 40890 RICE PM or 21456 IR PH. (W8462)A

COLLECTION LEADER (BIRD GROUP)

The Zoological Museum

Tring, Herts

In the field of life and earth sciences, the Natural History Museum is a major international institute, researching and curating collections numbering over 66 million items. We are recognised as a world-leader in the presentation of natural history to the general public through exhibitions.

Our bird collections are housed in a purpose-built complex next to the Zoological Museum in Tring and we are now looking for a Collection Leader to play an important role in the development of all aspects of the collection's management.

Leading and working alongside a team of five curators, you will be responsible for planning and implementing the Bird Group's curation programme, its Advisory Service and loan system, and a collection-based research programme.

In addition to a relevant degree and post-graduate experience in ornithology, this post calls for good skills in managing people and resources, and the ability to represent the Museum nationally and internationally on ornithological matters.

Starting salary will be in the range £21,905 - £26,121 and benefits include generous holidays and a non-contributory, index-linked pension scheme.

For further details and an application form (to be returned by 24 May 1991) write to Recruitment & Assessment Services, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 468551 (answering service operates outside office hours).
Please quote ref: B/1026/92.

An equal opportunity employer

THE NATURAL HISTORY MUSEUM

(6217)A

St George's Hospital Medical School

University of London

RESEARCH ASSISTANT 1B

Public Health Laboratory

Applications are invited for the post of Research Assistant for a Birthright funded study of the diagnosis of congenital toxoplasmosis. this is a three year full-time post with duties to include practical experimental techniques such as immunoblotting, PCR and serological methods. Work will be carried out in the toxoplasmosis reference laboratory under the direction of the grant holder, Dr Richard Holliman. Applicants should have a good first degree in Biological Science or a related subject. The successful candidate would be expected to register for a PhD. The salary will be in the range £13,166-£15,262 per annum (inclusive of London Allowance) on the R1B scale depending on age, qualifications and experience. Informal enquiries can be made to Dr Holliman on 081 672 9944 ext 55673.

Further details and an application form from the Personnel Officer, St George' Hospital Medical School, Cranmer Terrace, London SW17 0RE, 081 784 2791 (24-hr ansaphone). Closing date: 6th June, 1991. Please quote reference 51/91. (6208)A

POSTDOCTORAL POSITION IN TRANSPLANTATION IMMUNOLOGY

Applications are invited for a postdoctoral scientist to join research into molecular mechanisms involved in transplantation immunology. Knowledge and experience with southern and northern blotting techniques, *invitro* translation and polymerase chain reaction are essential. Familiarity with transplantation immunology is preferred. Excellent laboratory facilities and collaborative opportunities will be made available. The position is initially funded for three years. Inquiries should include a CV and three references.

James R Baker, Jr, MD, c/o Theresa Wagner, University of Michigan Medical Center, Immunogenetics Research Laboratory, 1150 W Medical Center Drive, MSRB 1, Room 1520, Ann Arbor, MI 48109-0666. (NW6704)A

DEPARTMENT OF PHARMACEUTICAL SCIENCES

LECTURESHIP IN MOLECULAR/ CELLULAR TOXICOLOGY

Applications are invited from graduates with excellent qualifications in a biological or pharmaceutical subject. The successful applicant is expected to have a strong interest and expertise in molecular or cellular biology and to contribute to the research in mechanistic toxicology in the Department. His/her modest undergraduate teaching duties will be in both the Pharmacy and Human Biology programmes and will include development of courses in toxicology.

Terms of appointment and salaries

***Lectureship** within and up to the maximum of the ranges:*
£12,086 to £16,755 per annum (Lecturer Grade A) or
£17,455 to £22,311 per annum, and exceptionally to £24,930 per annum (Lecturer Grade B).
Salaries are currently under review.

Appointment will be for a period of three years initially, with the possibility of renewal or subsequent transfer to a continuing appointment.

Application forms and further particulars may be obtained from the Personnel Officer (Academic Staff), quoting Ref. No. 9113/6, Aston University, Aston Triangle, Birmingham B4 7ET. Tel: 021-359 0870 (24-hour answerphone). Fax: 021-359 6470. Closing date for the receipt of applications: 31st May, 1991.

(6213)A

ASTON UNIVERSITY

UNIVERSITY OF OXFORD
SIR WILLIAM DUNN SCHOOL OF PATHOLOGY
Research Assistant
Academic-related research staff, grade 1A; Salary £13,495-£18,165

A postdoctoral research assistant is required to join Professor Brownlee's group seeking to characterise the DNA signals and the transcription factors which control human clotting factor IX expression in the liver. Previous experience of studies on gene regulation would be an advantage. The post is available from July 1991 until 31 October 1993, supported by the Wellcome Trust, and includes an enhancement premium of three incremental points. Applicants should apply as long as they are available before October 1991. Full details on request from Professor Brownlee (0865 275559).

Applications in writing, together with c.v. and names and addresses of two referees, to The Administrator, Sir William Dunn School of Pathology, South Park Road, Oxford OX1 3RE. Closing date for applications 31 May, 1991. *The University is an equal opportunity employer.* (6201)A

UNIVERSITY OF WALES COLLEGE OF MEDICINE
Department of Medical Biochemistry
RESEARCH OFFICER IN MOLECULAR BIOLOGY

A three-year research post, funded by the Natural Environment Research Council as part of the GAMES initiative, is available to work on the molecular biology of bioluminescence as an environmental indicator. This is a collaborative project with The Institute of Oceanographic Sciences. The successful applicant will join an active team using protein engineering to investigate the molecular biology and molecular evolution of intracellular signalling and bioluminescence.

Candidates should have a degree in biochemistry or another biological discipline. The post is also suitable for someone graduating this year. The successful applicant may have the opportunity of registering for a higher degree.

Salary on the scale for University Research and Analogous Staff Grade IB at a starting salary of £11,399 per annum (under review).

Further details (quoting Ref No M46/2/48) available from the Personnel Office, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN (Tel: 0222/742296) to whom applications in the form of a curriculum vitae with the names and addresses of two referees should be submitted by 31st May, 1991. (6214)A

UNIVERSITY OF NOTTINGHAM
Department of Botany in collaboration with Rothamsted Experimental Station
POSTGRADUATE RESEARCH ASSOCIATE AFRC Funded

Applications are invited for the above position, which is available immediately for 2 years, to work on the genetic manipulation of *Tapesia yalundae*, the sexual stage of the cereal eyespot pathogen, with a view to furthering our understanding of fungicide resistance and the relationship between wheat and rye pathotypes. The project offers scope for molecular biological approaches as well as field studies to assess the importance of the sexual stage in the generation of variation in field populations. Salary in the range £12,792 to £14,744 according to experience. Applications, including names, addresses and telephone and fax numbers of three referees should be sent to Mrs Sheila Redman, Department of Botany, University of Nottingham, Nottingham NG7 2RD to arrive not later than 24 May. Informal enquires about the position may be made to **Professor John Peberdy (Tel 0602 484848 ext 3650) or Dr John Lucas (Tel 0602 484848 ext 3316).** (6219)A

UNIVERSITY OF KENT AT CANTERBURY

THE BIOLOGICAL LABORATORY

Postdoctoral Research Fellow
Research Associate

Applications are invited for two three year posts funded by the MRC. The project directed by Dr S Jarvis, will (1) characterise and study the regulation of sodium-dependent nucleoside and nucleobase transport in renal membrance vesicles and cultured epithelial cells, and (2) clone the Na^+/nucleoside co-transporters. Applicants for the post of Research Fellow (A91/49) should have a doctorate and ideally experience in molecular biology techniques. Knowledge of animal cell culture would be advantageous. For the post of Research Associate (Ref A91/50) applicants should have or expect to obtain this summer a BSc or equivalent in a relevant biological science subject. The appointee may be able to register for a PhD. Salaries in the range £11,399-£13,495 pa.

Application forms and further particulars are available from The Personnel Office, The Registry, The University of Kent at Canterbury, Canterbury, Kent CT2 7NZ. Tel: (0227) 764000 ext 3915. Please quote the appropriate reference number. Closing date: 31st May, 1991.

An Equal Opportunities Employer. (6183)A

TWO TENURE-TRACK POSITIONS at the ASSISTANT PROFESSOR LEVEL

in molecular biology

are available in the Department of Biology at the University of Victoria. These positions are part of a new program in environmental health sciences and successful candidates will be associated with a planned Centre for Environmental Health. Current research efforts in this area are in DNA repair, mechanisms of mutagenesis and monitoring mutations in humans. Successful applications are expected to develop independent research programs, to participate in undergraduate and graduate teaching in the Department of Biology.

Each applicant should submit a curriculum vitae and the names of three referees to: Dr Louis A Hobson, Chair, Department of Biology, University of Victoria, Victoria, British Columbia, V8W 2Y2.

The deadline for receipt of applications is 31 July 1991.

In accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents. The University of Victoria is committed to an employment equity program. Women are particularly encouraged to apply.

(NW6702)A

OXFORD UNIVERSITY PRESS

Senior Editor

Mathematics

Based in Oxford, the person appointed will be expected to take full responsibility for the continuing success of a well established and prestigious list of academic books, scholarly monographs, and university texts, to maintain contact with the mathematical community worldwide, to continue a vigorous programme of acquisitions in the USA and in Europe, and to contribute to the development of the publishing programme in a broad spectrum of physical and biological sciences. A degree in mathematics is required as are several years publishing experience at a responsible level. Opportunities may develop for a suitable candidate to take a broader managerial role within the department. An attractive salary and benefits package will be offered.

Please write, by 24th May 1991, with up to date c.v. and current salary to:

Mr D. C. Moody, U.K. Personnel Director, Oxford University Press, Walton Street, Oxford OX2 6DP (6211)A

UCL

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF PHYSIOLOGY

Post-Doctoral Position In Neuroscience

Funded by the Wellcome Trust, to work with David Attwell & Peter Mobbs using patch-clamp and fluorescence techniques to study the electrical properties of neurones and glial cells, with an emphasis on the functions of glutamatergic synapses and the blood-retina barrier (see Nature 328, p522; 332, p451; 348, p443). Electrophysiological experience essential. Send CV with names of referees to David Attwell, Department of Physiology, University College London, Gower Street, London WC1E 6BT. Telephone 071 380 7342.

U.C.L. is an Equal Opportunities Employer.

(6206)A

POST-DOCTORAL POSITION MOLECULAR NEUROBIOLOGY

Applications are invited for this 2-year, SERC funded position which is available immediately. The grant is held jointly by Dr J A Davies, Dr S E Blackshaw and Dr K Kaiser, Departments of Genetics and Cell Biology, Glasgow University.

The project involves the construction of cDNA libraries from small numbers of identified leech neurones, followed by subtraction hybridisation to isolate molecules unique to particular cells. Experience in Molecular Biology is essential.

Applications with full c.v. together with the names of 2/3 referees should be sent to Dr J A Davies, Institute of Genetics, University of Glasgow, Church Street, Glasgow G11 5JS. Tel: 041-339 8855, ext. 5654/6234. Fax: 041-330 5994. (6189)A

INSTITUTE OF ZOOLOGY
ACADEMIA SINICA
Taipei, Taiwan, ROC

FACULTY POSITIONS

The Institute of Zoology, Academia Sinica is seeking to recruit five outstanding individuals to faculty positions in the area of basic research related to (modern) zoological science. **The faculty positions are Research Fellow, Associate Research Fellow and Assistant Research Fellow.** Specific research areas could be in Molecular Biology, Cell Biology, Biochemistry, Molecular Genetics, Immunology, Chemical Ecology, Developmental Biology, Endocrinology, Protein Chemistry, Population Genetics, Evolutionary Biology, Marine Molecular Biology, and Aquatic Zoology. Positions are available immediately, and rank will be commensurate with qualifications.

Candidates should have a Ph.D. degree and/or research experience. Successful candidates will be expected to establish well-funded research programs and should devote fifty per cent effort to join one of the main research programs of the institute.

Positions for Post-Doctor Fellowship are also available.

If you are interested in these positions, please send your Curriculum Vitae, a statement of research plans, a list of publications plus copies of selected recent publications, and two letters of reference to:

Jen-Leih Wu. Ph.D., Research Fellow and Director, The Institute of Zoology, Academia Sinica, Taipei, Taiwan 11529, Republic of China (ROC)
Fax: 886-2-785-8059

The Institute Faculty Position is preferring candidates with ROC citizenship or Oversea Chinese status. (W8466)A

DEPARTMENT OF PLANT SCIENCES UNIVERSITY OF OXFORD DEPARTMENTAL DEMONSTRATORS

Applications are invited for two Departmental Demonstratorships to be appointed from October 1991 within the following fields:

1. **Experimental mycology or biochemical and molecular plant pathology;**
2. **Plant systematics and evolution.**

The salary will be in the range £11,399–£15,444 and both appointments will be tenable for a period of up to three years in the first instance with the possibility of renewal for a further and final period of three years. The persons appointed should have a Ph.D. by the time of their appointment and will be required to undertake teaching and are encouraged to develop a research programme.

Four copies of applications, including c.v., research interests and experience, together with the names of two referees, should be sent to Professor C. J. Leaver, Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB to arrive not later than 1 June 1991.

Further particulars may be obtained from Professor Leaver by telephoning 0865 275143. (6202)A

KING'S COLLEGE LONDON

Division of Biomolecular Sciences

We have a three-year Wellcome-funded post-doctoral vacancy to work with Dr Keith Dudley on a project to identify the DNA binding-sites for a new zinc-finger protein expressed exclusively in the male germ cells. Starting salary up to £18,522 inclusive. The post will be located in a new developmental biology initiative between this Division and the Anatomy Department of the Biomedical Sciences Division, in the Drury Lane Laboratories.

Applications, to include a curriculum vitae and the names and addresses of two referees, should reach **Mr D M Drummie, Divisional Administrator, King's College London, 26-29 Drury Lane, London WC2B 5RL, by 3 June 1991. Informal enquiries, and further details: Dr Dudley on 071-333 4490.** (6203)A

PROFESSOR OF MARINE CHEMISTRY

UNIVERSITY OF GÖTEBORG, SWEDEN

Applications are invited for a newly created Chair of Marine Chemistry at the Department of Analytical and Marine Chemistry, University of Göteborg and Chalmers University of Technology.

The duties of the professor are his own scientific research, teaching at all levels, supervision of graduate students and certain administrative responsibilities.

The subject of the chair is Marine Chemistry without any further specification and the professor will be selected entirely on his own merits. The professor will work in a joint department of Analytical and Marine Chemistry (Daniel Jagner holds the chair in Analytical Chemistry).

Further information concerning the position can be obtained from Professor Daniel Jagner, Dean of the School of Chemistry, University of Göteborg, S-412 96 Göteborg, Sweden. Telephone +46 31 72 27 71, telefax +46 31 72 27 85.

Applications, in four sets, should be directed to the Chancellor of the University of Göteborg and mailed to the Registrator, University of Göteborg, Vasaparken, S-411 24 Göteborg, Sweden.

The application should contain
(i) a resumé of the applicant's research and teaching activities
(ii) a curriculum vitae including a list of published papers, submitted or unsubmitted manuscripts, books, etc.
(iii) reprints of the work referred to by the applicant.

The resumé should contain a list of those publications or other work which the applicant considers most relevant (at most 20 titles). Copies of these should be included in all four sets of applications. Additional publications may be quoted, but for these one copy is sufficient.

Closing date: June 11, 1991.

Please quote reference No E311 1086/91.
Reprints etc, not available at the closing date, may be submitted within three weeks thereafter, provided that they are listed in the curriculum vitae. (W8469)A

TEMPORARY LECTURESHIP

DEPARTMENT OF PHARMACOLOGY
UNIVERSITY OF CAMBRIDGE

A position is available for 2/3 years tenable from 1 October 1991. The successful applicant will be required to take part in teaching and should have at least three years postdoctoral experience, with an active, on-going research programme relevant to Pharmacology.

Salary on the Lecturer Scale (£12,086–£23,819 according to age and experience.

Application should be sent to Mrs A Horn, Department of Pharmacology, Tennis Court Road, Cambridge, CB2 1QJ. (6173)A

UNIVERSITY OF NOTTINGHAM
Department of Obstetrics and Gynaecology

POST-DOCTORAL RESEARCH ASSISTANT IN VASCULAR REACTIVITY

Applications are invited for a post-doctoral research assistant to work with Professors F Broughton Pipkin and I R Johnson on aspects of the control of vascular reactivity in the uterine vasculature in normal and hypertensive human pregnancy. The work will particularly relate to the identification and characterisation of uterine angiotensin II receptors. Previous experience with receptor assays would be an advantage but is not essential. Applicants should have an Honours Degree, preferably in Physiology, Biochemistry or a related science, and a PhD or equivalent research experience. The position is for 2 years and the salary will be at an appropriate point on the Research Staff I A scale (£11,399-£18,165 pa under review) depending on qualifications and experience.

For further details telephone or write with a curriculum vitae and names of three referees to: Professor F Broughton Pipkin, Department of Obstetrics and Gynaecology, East Block, University Hospital, Nottingham NG7 2UH. (6220)A

UNIVERSITY OF SOUTHAMPTON

RESEARCH ASSISTANT (1B)

The above position is available in the areas of Obstetrics/Gynaecology and Physiology within the Faculty of Medicine to study paracrine control of human ovarian function using cellular and molecular biology techniques. Candidates should have (or expect to obtain) a good degree in the Life Sciences and be interested in registering for a higher degree. Funding is presently available to appoint for 2 years on the initial point of the scale (starting salary: £11,399-12,086 pa under review) with a reasonable expectation of further support to complete postgraduate studies.

The supervisors (Drs M C Richardson, M J Peddie and Professor E J Thomas in the 2 collaborating departments) may be telephoned for discussion (0703 796044). Applications (2 copies), including a full cv and the names, addresses and telephone numbers of 2 referees, should be sent to **M/282, The Personnel Department, University of Southampton, Highfield, Southampton SO9 5NH, by 24 May 1991.**

Working for Equal Opportunities. (6222)A

UNIVERSITY OF SHEFFIELD

Department of Biomedical Science

RESEARCH ASSISTANT (GRADE B)

Applications are invited for a three year post funded by the British Diabetic Association. The project will investigate the receptor-operated control of ion channels in pancreatic β-cells using patch-clamp techniques.

Candidates should have or expect to receive a good honours degree in Physiology, Biochemistry or a related discipline.

The successful applicant will be expected to register for a higher degree. Salary will be on the IB scale for Research & Analogous Staff (from £11,399 pa).

Applications, including a CV and the names/addresses of two referees to: Dr M J Dunne, Department of Biomedical Science, University of Sheffield, Western Bank, Sheffield S10 2TN. Informal enquiries welcome. Please quote Ref: R1112/G.

An Equal Opportunity Employer. (6228)A

Washington
WASHINGTON UNIVERSITY IN ST. LOUIS

TENURE TRACK ASSISTANT PROFESSOR

MOLECULAR BIOLOGY AND PHARMACOLOGY

Washington University School of Medicine is recruiting an individual at the level of Assistant Professor in the tenure track. Outstanding scientists working within the broad area of molecular biology will be considered with an emphasis on those who have a strong background in mammalian genetics and who are expert at using transgenic mice to examine fundamental questions in gene regulation and function.

Interested applicants should forward a curriculum vitae, a short summary of research plans, and the names and addresses of three references to: Search Committee, c/o Jeffrey Gordon, Head, Department of Molecular Biology and Pharmacology, Box 8103, Washington University School of Medicine, 660 South Euclid Avenue, St Louis, MO 63110.

Washington University is an Affirmative Action/Equal Opportunity Employer. (NW6687)A

THE UNIVERSITY OF CALGARY

FACULTY POSITION IN IMMUNOLOGY RESEARCH

The University of Calgary Faculty of Medicine Immunological Sciences Research Group invites applications for a full-time academic position at the Assistant Professor level for research in inflammation.

Qualifications include a minimum of two years' postdoctoral research experience in inflammation and/or cytokine networks. Preference will be given to the candidate with interests in inflammation as it relates to mucosal immunology, neuroimmunology, oncology or transplantation immunology. The Immunological Sciences Research Groups has fourteen members with diverse interest in immunology and inflammatory diseases.

The selected applicant must compete successfully for salary support from the Alberta Heritage Foundation for Medical Research and/or other provincial and national agencies.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. The University of Calgary has an Employment Equity Program and encourage applications from all qualified candidates, including women, aboriginal people, visible minorities, and people with disabilities.

Please submit a curriculum vitae, names of three referees, and an outline of research interests and intent by August 31, 1991, to: **Dr. Dean Befus, Chair, Immunological Sciences Research Group, The University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta T2N 4N1.** (NW6695)A

UNIVERSITY OF MIAMI SCHOOL OF MEDICINE,

DEPARTMENT OF NEUROLOGY

NEUROIMMUNOLOGIST

Applications are invited from MD or PhD neuroimmunolgists at the Associate or Full Professor level to direct the Neuroimmunology Section of the Department of Neurology. Tenure decisions will be based upon candidate's qualifications. A VA/MC position may be available. Applicants should have established laboratory research programs and clinical interests. MD's must be Board eligible /certified and eligible for a Florida Medical License. This is an exciting growth position in a department with extensive existing neuroscience research programs, including MS, AIDS, and the neuromuscular diseases. Collaborative opportunities in UMSM are excellent, with extensive research groups in immunology, virology, and molecular biology.

Applications with cv, names of 3 references, and copies of 5 recent publications to: Walter G Bradley, DM, FRCP, Professor and Chairman, University of Miami School of Medicine, Department of Neurology (D4-5) PO Box 016960, Miami, Florida 33101. The University of Miami is an Equal Opportunity/Affirmative Action Employer. (NW6558)A

Senatsverwaltung für Wissenschaft und Forschung
Der Senator als Vorsitzender der Personalkommission der Freien Universität Berlin

BERLIN

DAHLEM KONFERENZEN

We are seeking a successor to the founding Director of the Dahlem Konferenzen, Dr. Silke Bernhard, who has had to resign because of ill health.

The Dahlem Konferenzen, founded in 1974, organise workshops to promote interdisciplinary cooperation between leading scientists from all parts of the world. The workshops, held four times a year in Berlin, focus chiefly on the environmental and biomedical sciences. They provide a limited number of participants with the opportunity for intensive discussion in areas at the forefront of comtemporary research. The Dahlem Konferenzen are funded by the Land Berlin, the Stifterverband für die Deutsche Wissenschaft and the Deutsche Forschungsgemeinschaft. Since January 1990 they have been a part of the Freie Universität Berlin.

The Director must be a highly qualified scientist with a Ph.D. or M.D. in natural or biomedical sciences and considerable postdoctoral research experience. He/she must be a proven organiser with the ability to lead and cooperate with a dedicated team. Fluent English and German are essential and experience with organising scientific meetings and in editing scientific publications would be an advantage.

The salary will be determined according to qualifications under the provisions of the German public service ordinances. Applications, including a photograph, CV and publication list, should be sent within four weeks to the Senator für Wissenschaft und Forschung, the chairperson of the Kuratorium of the Freie Universität Berlin, I E, Bredtschneiderstr. 5, W-1000 Berlin 19, quoting reference number 32/91. (W8453)A

UNIVERSITY OF MIAMI SCHOOL OF MEDICINE,

DEPARTMENT OF NEUROLOGY

NEUROVIROLOGIST

Applications are invited from MD or PhD neurovirologists at the Associate or Full Professor level to direct the new Neurovirology Section of the Department of Neurology. Tenure decisions will be based upon candidate's qualifications. A VAMC position may be available. Applicants should have an established laboratory research program in neurovirology, preferably concentrating on retroviruses. MD's must be Board eligible/certified, and eligible for a Florida Medical License. This is an exciting growth position in a department with extensive existing neuroscience research programs, including an AIDS Center, and Neuroimmunology Program. Collaborative opportunities in UMSM are excellent, with extensive research groups in virology, immunology, and molecular biology.

Applications with CV, names of 3 references, and copies of 5 recent publications to: Walter G Bradley, DM FRCP, Professor and Chairman, Department of Neurology (D4-5), PO Box 016960, Miami, Florida 33101. The University of Miami is an Equal Opportunity/Affirmative Action Employer. (NW6557)A

UNIVERSITY OF MIAMI SCHOOL OF MEDICINE
DEPARTMENT OF NEUROLOGY
MOLECULAR NEUROBIOLOGIST

Applications are invited from MD or PhD molecular biologists and molecular geneticists at the Assistant or Associate Professor level, interested in neurological and/or neuromuscular diseases. Tenure decisions will be based upon candidate's qualifications. MDs must be Board eligible/certified and eligible for a Florida Medical License. A VAMC appointment is available if desired. Applicants should have an established research program using molecular genetics techniques to investigate neurological function and disease. This is an exciting growth position in a department with extensive existing neuroscience research programs. Collaborative opportunities in UMSM are excellent, with several large molecular biology and molecular genetics groups.

Applications with CV, names of 3 references, and copies of 5 recent publications to: Walter G Bradley, DM, FRCP, Professor and Chairman, University of Miami School of Medicine, Department of Neurology (D4-5), PO Box 016960, Miami, Florida 33101. The University of Miami is an Equal Opportunity/Affirmative Action Employer. (NW6556)A

The Medical College of St. Bartholomew's Hospital (University of London)

POST DOCTORAL RESEARCH ASSISTANT(£13,166-£17,793 pa)
RESEARCH TECHNICIAN (£10,564-£13,364 pa)

The above positions are available to research the relationship between hepatocyte de-differentiation and the expression of proto-oncogenes/transcription factors. Experience with Northern/Western blotting, nuclear transcription rate assays, S1 oligonucleotide protection assays, PCR or transfection techniques is essential. Both appointments are available for up to three years. The technical vacancy may suit an experienced technician who is looking for career advancement through registration for a higher degree.

Salary will be on 1A or the MLSO 1 scale depending on age and experience.

Informal enquiries should be made to Dr Alan J Paine (Tel. 071 601 8610), or by writing to him at the Department of Toxicology, St Bartholomew's Hospital, Dominion House, 59 Bartholomew Close, London EC1 7ED.

Working towards Equal Opportunities.

Closing date: 7th June 1991 (6186)A

PhD Student in Molecular Biology at the University of Basel

At the lab of molecular endocrinology our main research topic is the regulation of IGF (insulin-like growth factor) activity at the molecualr and cellular level. The work will consist of "classic" molecular biology and its application in different cellular systems. In our team strong personal initiative and participation in formulating new projects will be encouraged. Positions can be fille don July 1 and October 1, 1991. Applications with full CV and the names of two referees should be sent to:

Dr Jürg Schwander, Department of Internal Medicine and Centre for Research, Kantonsspital, 4031 Basel, Switzerland. Telefax (41) 61 35 64 33. (W8465)A

THE UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF PHARMACOLOGY
TWO 3 YEAR POSTDOCTORAL RESEARCH FELLOWS

To work on the molecular structures and regulation of vertebrate EAA receptors (see TIPS, 1990: 11, 504-511). The projects will involve a variety of techniques including autoradiography, radioligand binding, receptor purification, protein modification and molecular biology.

Maximum starting salary £14,038.

Informal enquiries to Dr Jeremy Henley on 021-414 4508.

Application forms (2 copies) returnable by 30 June 1991 (including a cv and list of publications) and further particulars available from the Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham B15 2TT. Tel. 021-414 6483 (24 hours). Quote ref M12203.

The University is an equal opportunities employer.

PHD STUDENTSHIP

An MRC quota award studentship starting in October 1991 is also available to work in one of the following areas: 1) neurochemistry of 5-HT and/or Angiotensin II receptors, 2) the *in vitro* electrophysiology and synaptic pharmacology of substantia nigra, or 3) the molecular characterisation of EAA receptors.

Requests for further information should be made to Dr Jeremy Henley on 021-414 4508. Closing date for receipt of applications 30 June 1991. (6181)A

KENNEDY INSTITUTE
Cell Enzymology Unit

A post-Doctoral Scientist or Research Assistant is required, for one year in the first instance, to work on a project supported by the Muscular Dystrophy Group. The aim is to study the differentiation and function of virus-immortalized muscle cells from normal individuals compared with those from patients with muscle disease. Experience of tissue culture and immunochemical procedures would be an advantage. Salary is at the appropriate point of Scale 1A or 1B, and will attract an enhancement premium at the post-Doctoral level.

Applications should be addressed to the General Secretary, Kennedy Institute of Rheumatology, 6 Bute Gardens, Hammersmith, London W6 7DW, and should include curriculum vitae and names and addresses of two referees. (6227)A

STUDENTSHIPS

UNIVERSITY OF EDINBURGH
Department of Biochemistry
SERC STUDENTSHIPS

Applications are invited for two SERC studentships to study for a PhD for 3 years from October 1st 1991. Research projects are available in the following areas:

Enzymology of bacterial cytochrome c peroxidase.

Membrane traffic and protein secretion in yeast.

Action of cholera toxin on intestinal cells.

Properties of vacuolar H^+ATPases.

Candidates should hold or expect to obtain a First or Upper Second Class Honours degree in a relevant subject. Informal enquiries should be made to David Apps or Alan Boyd, postgraduate tutor, (telephone 031 650 3722 or 650 3724).

Applications including a full cv and the names of two academic referees should be sent to Dr David K Apps, Department of Biochemistry, University of Edinburgh Medical School, Hugh Robson Building, George Square, Edinburgh EH8 9XD. (6195)F

UNIVERSITY OF YORK
BIOLOGY DEPARTMENT

RESEARCH STUDENTSHIP IN MEMBRANE TRANSPORT & SIGNAL TRANSDUCTION

An AFRC-funded graduate studentship is available, starting in September 1991. The project involves characterization of plasma membrane ion channels in fungi, with particular emphasis on plant pathogens.

The studentship is a collaborative one between Dr Dale Sanders (York), Dr Graham Dixon (Dow Elanco Ltd, Letcombe Regis) and Dr Colin Brownlee (Marine Biological Association, Plymouth). The student will have the opportunity for training in a wide variety of contemporary cell biological techniques, including patch clamping and fluorescent imaging of intracellular ion activities.

It is anticipated that the student will spend at least the first few months of the project at Dow Elanco, developing methodology for isolation of protoplasts suitable for patch clamping. Thereafter, the student will be based at York, where (s)he will join a large research group active in various aspects of membrane transport and signal transduction in plants and fungi. There will, however, be the opportunity to conduct imaging experiments at Plymouth.

The stipend from the AFRC will be £5,000 p.a., and this will be supplemented by Dow Elanco by a further £1,250 p.a.

There is no closing date for applications. Applicants should have, or expect to obtain at least an upper second class honours degree in a biological subject and should send three copies of their curriculum vitae to Dr Sanders as soon as possible. Informal enquiries concerning the studentship are encouraged, and can be made to Dr Sanders (0904-432825, direct line) or to Dr G. Dixon (0235-772900, switchboard. (6178)F

INSTITUTE OF PSYCHIATRY
Department of Psychiatry

MRC Research Studentship

Applications are invited for training in one of the research areas listed below, leading to entry for the MPhil or PhD degree of the University of London. The studentship will support a recent graduate (II i or above) for 3 years from September 1991.

RESEARCH AREAS:

(1) Behavioural pharmacology; models of drug addiction and of multi-drug abuse.
(2) Genetic susceptibility to alcoholic liver damage.
(3) Body image changes and dreaming linked with brain damage and psychiatric illness.
(4) Disturbed eating behaviour and physiology in the Prader-Willi syndrome (genetic obesity).
(5) Neuropsychological correlates of hallucinations seen in alcohol withdrawal.

Applications should take the form of a brief cv with the names of two referees and a covering letter explaining why you are applying for the studentship and should be sent to the **Personnel Office, Institute of Psychiatry, De Crespigny Park, London SE5 8AF. Please state the research areas in which you would like to be considered for training and quote reference 91/R11 (closing date June 14th).** (6207)F

UNIVERSITY OF NOTTINGHAM
Department of Physiology and Environmental Science
SCHOOL OF AGRICULTURE

PHD STUDENTSHIP IN ANIMAL PHYSIOLOGY

Paracrine Control of Folliculogenesis in Pigs

An AFRC Research Studentship is available, from October 1 1991, to investigate the intra-ovarian control of follicular development in pigs, in both European and the highly prolific Chinese Meishan breeds. The relationships between follicles in this polytocous species and the role of regulatory factors produced by the ovary on follicle development will be studied using both cell culture systems and physiological approaches. The successful applicant will join an active Group with considerable expertise in this area and will be trained in whole animal physiology, cell and tissue culture as well as radioimmunoassay and histological techniques.

Candidates should hold, or expect to gain, at least an Upper Second Class Honours degree in Animal Science, Physiology, Biochemistry or a related subject. For further details and application submissions (containing names of two academic referees) contact **Dr Morag Hunter, Department of Physiology and Environmental Science, University of Nottingham, Sutton Bonington, Loughborough, Leics LE12 5RD (Tel: 0602 484848 ext 8283).** (6221)F

Leicester University

Department of Botany
School of Biological Sciences

RESEARCH STUDENTSHIPS IN PLANT PHOTOBIOLOGY

Applications are invited from candidates who hold, or expect to achieve, at least an Upper Second Class honours Degree in an appropriate subject for the following research council studentships.

1. Photomorphogenesis of the *ein* mutant of *Brassica rapa*. (Supervisor: Dr G C Whitelam - AFRC).
2. Photosynthetic characteristics and assimilate partitioning in transgenic plants expressing introduced phytochrome genes. (Supervisor. Professor H Smith - AFRC).
3. Evolution and expression of photoreceptor genes in relation to shade adaptation strategy. (Supervisor. Professor H Smith - NERC).

The successful candidates will work within a group using molecular biological, biochemical and physiological techniques to investigate the function, action and adaptive significance of the members of the photochrome gene family. Further details may be obtained form the named staff.

Applications with a full CV and the names and addresses of two academic referees should be sent, as soon as possible, to **The Secretary, Department of Botany, University of Leicester, Leicester LE1 7RH. Applications by phone (0533 523381) or fax (0533 522791) will be welcomed.** (6191)F

UNIVERSITY OF GLASGOW
Departments of Biochemistry and Botany

POSTGRADUATE STUDENTSHIPS

Four studentships are available to work on the following projects from October 1991:

1. AFRC Postgraduate Studentship with Dr J G Lindsay and Prof R J Cogdell to carry out immunological and regulatory studies on plant 2-oxoacid dehydrogenase multienzyme complexes.
2. AFRC Postgraduate Studentship with Dr G I Jenkins to work on the isolation and characterisation of mutants of *Arabidopsis* altered in signal transduction for light-induced responses.
3. SERC CASE Studentship with Dr G I Jenkins and Dr W Schuch (ICI Seeds, Bracknell) to study the structure and expression of cold-induced genes in crop plants.
4. SERC Postgraduate Studentship with Dr H G Nimmo to work on molecular characterisation of a plant protein kinase.

The successful applicants will carry out research for three years leading to a PhD degree. The Departments have very good facilities for research and place a strong emphasis on postgraduate training. The conditions and levels of the awards are as determined by the appropriate Research Councils. The CASE student will receive a supplement from ICI Seeds and will spend a period of time in their research laboratories.

Applicants who must have or expect to obtain an upper second class honours degree in molecular biology, biochemistry, plant science or an equivalent subject, should send a full CV including the names of two referees to the appropriate supervisor at the Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ. The deadline for applications is 22 May, 1991. Further information can be obtained by telephoning Dr Jenkins, Dr Lindsay or **Dr Nimmo on 041 339 8855 ext 5906, 4720 or 4721 respectively.** (6218)F

UNIVERSITY OF ST ANDREWS
Department of Biology & Preclinical Medicine
RESEARCH STUDENTSHIPS

Research Studentships are available on the following research topics, commencing October 1991:

1. "The role of urotensins in elasmobranch fish" (Supervisor Dr N Hazon).
2. "Environmental physiology of foraging and reproduction in solitary bees and other insects" (Supervisor Dr P Willmer).
3. "Complement Phylogeny" (Supervisor Dr V Smith).
4. "Dietary modulation of atrial natriuretic peptide receptors" (Supervisors Drs J Aiton & G Cramb).
5. "Vasodilator properties of novel-nitric-oxide donor drugs (Supervisor Dr F W Flitney).
6. "Modulation of neurotransmitter release by presynaptic autoreceptors" (Supervisor Dr R Pitman).
7. "Plant molecular diversity and species relationships (Supervisors Dr R Ingram).
8. "Influence of temperature on fish muscles and locomotion" (Supervisor Prof. I A Johnston).

These studentships will be financed by NERC, SERC, Maitland-Ramsay Trust and the University of St Andrews. To obtain further details on individual topics and an application form, please write directly to the individual supervisor at the following address, giving the names and addresses of two academic referees: **Department of Biology & Preclinical Medicine, University of St Andrews, St Andrews, Fife, Scotland.** (6176)F

YAMANOUCHI RESEARCH STUDENTSHIP

A PhD studentship will be available in the Department of Medical Oncology, University of Manchester, from October 1991. The successful candidate will be joining a team interested in the role of the extracellular matrix in neovascularisation and will be using a variety of techniques in biochemistry, cell and molecular biology. Salary will be £8,400 in the first year, with the appropriate increments for the second and third years.

Applications, including a full CV and names and addresses of three referees, should be sent to Dr A M Schor, CRC Department of Medical Oncology, University of Manchester, Christie Hospital and Holt Radium Institute, Wilmslow Road, Manchester, M20 9BX, UK, from whom further information is available (tel. 061 445 8123, ext. 432). Closing date: 21st June, 1991. (6159)F

IMPERIAL COLLEGE
(University of London)
RESEARCH STUDENTSHIPS

Applications are invited to join research groups in the Centre for Fusion Studies and the Energy Systems Analysis Sections at Imperial College. The work of these groups covers a range of subjects in the areas of Nuclear Fusion Technology, Electromagnetic Analysis, and Radiation Transport. A common theme to the research is the development and application of advanced computational techniques, including novel applications of the finite element and boundary integral equation methods, and their implementation on a large parallel processing computer. Much of the research is interdisciplinary, involving collaboration between the Mechanical Engineering, Electrical Engineering and Physics Departments of Imperial College; support for the work is from both Research Councils and a variety of industrial sources.

Suitable candidates could come from a range of backgrounds; the first degrees of current researchers include mathematics, physics, electrical and mechanical engineering. More important is enthusiasm, and a strong academic background. In the first instance please telephone for an informal discussion of the range of opportunities, or address applications to: **Dr S P Walker, Deputy Director, Centre for Fusion Studies, Imperial College, London SW7 2BX, 071 225 8630.** (6182)F

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF ZOOLOGY
SERC-CASE STUDENTSHIPS

Two CASE studentships are available from October 1991. Applications are invited from candidates who have, or expect to obtain, a good honours degree [upper second or first] in a biological subject. Interests in parasitology, immunology, biochemistry and cell biology would be relevant.

1. *Development of a faecal antigen ELISA for detection of gastrointestinal nematode infections.* Studentship between Dr J M Behnke and the MAFF, Central Veterinary Laboratory, Weybridge.
2. *Regulation of eosinophil responses to inflammatory stimuli.* Studentship between Professor D Wakelin and Rhone-Poulenc Rorer, Central Research, Dagenham Research Centre.

Further details are available on request. Please send applications, giving full c.v. and names of two referees, to the Departmental Secretary, Department of Zoology, University of Nottingham, Nottingham NG7 2RD by 31 May 1991. (6175)F

UNIVERSITY OF LONDON
Queen Mary and Westfield College
School of Biological Sciences
PhD STUDENTSHIPS IN FUNGAL MOLECULAR GENETICS

Two studentships, one funded by SERC and one funded by AFRC, are available to work on the molecular manipulation of the mating type genes of the mushroom *Coprinus cinereus*. These genes encode proteins with a HOMEODOMAIN, the hallmark of proteins shown to regulate development in mammals, insects and plants. Because we can transform genes easily, the fungal system allows us the unique opportunity to dissect the function of such proteins.

Tenable from 1 October 1991. Graduates will require a first or upper second class honours degree and will be eligible for a travel and book allowance of £500 per year. Applications to: **Professor L. A. Casselton, School of Biological Sciences, Queen Mary and Westfield College, Mile End Road, London E1 4NS.** (6187)F

UNIVERSITY OF LEICESTER
DEPARTMENT OF MICROBIOLOGY
MRC STUDENTSHIP

Applications are invited for an MRC Postgraduate Studentship leading to a degree of Ph.D work on a project investigating the mechanisms by which macrophages kill *Mycobacterium tuberculosis*. The studentship is for three years from October 1991. Applicants should have or expect to obtain a first or upper-second class honours degree in a biological subject.

Applications, including the names of two referees, should be sent to Dr P. W. Andrew, Department of Microbiology, PO Box 138, Medical Sciences Building, University Road, Leicester LE1 9HN. Further details also can be obtained from Dr Andrew, tel: 0533 522941. (6174)F

AFRC INSTITUTE OF ANIMAL PHYSIOLOGY AND GENETICS RESEARCH
Roslin, Midlothian, EH25 9PS
RESEARCH STUDENTSHIPS
EDINBURGH

Applications are invited now for a research studentship tenable for 3 years from 1st October 1991. The successful applicant will join the Bone Biology Programme and will work on a project investigating the role of Bone Morphogenetic Proteins and related growth factors in the regulation of osteoblast differentiation and function. Techniques will include in situ hybridization and tissue culture.

Applicants should have (or expect to obtain) a 1st or 2.1 honours degree in Biochemistry, Molecular or Cellular Biology or a related subject. The standard conditions of the AFRC will apply, including the newly enhanced stipend.

Applications including a full CV naming two referees should be sent as soon as possible to Dr. Houston or Dr. Thorp at the above address. Informal enquiries. Tel. 031-440 2726. (6177)F

STUDENTSHIPS/FELLOWSHIPS

UNIVERSITY OF BATH
School of Pharmacy and Pharmacology
Medicinal Chemistry Group
SERC PhD Studentships

An opportunity to undertake research leading to the degree of PhD, funded by SERC, is available on a project concerned with the semi-synthesis and pharmacological characterization of novel antagonists for nicotinic acetylcholine receptors. The mode of action of these plant alkaloids is to be determined in this Studentship supervised by Dr I S Blagbrough, Dr M G Rowan, and Professor B V L Potter.

Candidates who hold or expect to obtain a I or 2i degree in pharmacy, chemistry, or the biological sciences, or those wishing to make informal enquiries should contact Dr I S Blagbrough (0225 826795) as soon as possible.

Other SERC (quota and CASE) awards are also available in the general areas of pharmacology, medicinal chemistry, and pharmacy within the School. Enquiries to Dr K I Williams (0225 826842) in the first instance. (6215)F

INTERNATIONAL CANCER RESEARCH FELLOWSHIP

The Hayashibara Foundation, a nonprofit-making organization within the Hayashibara Group, announces a

FELLOWSHIP PROGRAM

Fellowship will be taken up at the Fujisaki Cell Center which is devoted to basic and applied research related to the problems in human cancer. The fellowship is normally made for 1 year and is renewable for up to 5 years. The fellowship will be expected to commence within 6 months of the announcement of the awards. No fixed deadline for application is set throughout the year.

The awards are for high quality research work in one of the three categories described below.

1. *Fundamental Leukemia-Lymphoma Research.*
2. *Cytokine-Lymphokine Research.*
3. *Hematopoietic Cell Lines.*

Qualified persons holding a PhD, MD or equivalent qualifications should make inquiry for additional information and application forms from Jun Minowada, MD, Director, Fujisaki Cell Center, 675-1, Fujisaki, Okayama 702, Japan. (W8174)E

UNIVERSITY OF LEEDS
CENTRE FOR NONLINEAR STUDIES STUDENTSHIP

A Cognitive Science/HCI Initiative studentship, to be held through the Department of Physiology, is available on:

The formation and maintainance of auditory space maps by neuronal networks.

The student will receive an interdisciplinary training in the Department of Applied Mathematical Studies, School of Computer Studies, and Department of Physiology.

Applicants should have either a good honours degree in applied mathematics, computer science, or theoretical physics, with a strong interest in nonlinear dynamics, cognitive science or neural networks; or a good training in one of the neurosciences with evidence of mathematical aptitude and a strong interest in cognitvie and computational neuroscience.

For further information contact Dr Arun Holden, Centre for Nonlinear Studies, The University, Leeds LS2 9JT. Tel: 0532 334251 Fax: 0532 334381 (mark for attention of Dr Holden, Physiology), e-mail phs6avh @leeds.cms1. (6216)F

MOREDUN RESEARCH INSTITUTE
POSTDOCTORAL VIROLOGIST
Ref: ADCA 491/1

A research fellowship is available for 3 years in a group studying enteric viruses of young animals. The appointee will be particularly concerned with characterising equine rotaviruses and their role in foal diarrhoea.

Salary and placement according to qualifications:

HSO £11586 - 16176
SSO £14381 - 20467
VRO £21905 - 26121

Further information can be obtained by phoning Dr. D. Snodgrass on 031-664 3262.

Applications with the names and addresses of 2 referees to the Personnel Officer, Moredun Research Institute, 408 Gilmerton Road, Edinburgh.

Closing date 31st May 1991. (6188)E

THOMPSON-MAYO FELLOWSHIP FOR BASIC RESEARCH ON ALCOHOL AND DRUGS OF ADDITION

Two fellowships available in 1991 at Mayo Foundation, Rochester, Minnesota, for studies of the biological effects of either alcohol or other drugs of addiction. The research may be conducted in any of the research laboratories of the Mayo Foundation. Applicants must have appropriate academic credentials (MD, PhD or equivalent degree, with or without postdoctoral training). Selection will be predicated on the qualifications of the applicant and will be made on a purely competitive basis by the Mayo Research Committee. Tenure of each fellowship will be two years. Salary and benefits will be determined by the successful candidate's experience but will be competitive with the salaries offered by the best fellowships available nationally at the Postdoctoral/Research Associate level.

Applications should be submitted to Dr. Steve Brimijoin, Chair, Personnel Committee of Research Committee, Mayo Foundation, Rochester, MN 55905 by June 1, 1991. Mayo Foundation is an affirmative action and equal opportunity educator and employer. (NW6698)E

KENDALL-MAYO FELLOWSHIP IN BIOCHEMISTRY

Mayo Foundation is now considering applications from highly qualified candidates for appointment as the Kendall Fellow in Biochemistry. This nationally competitive award, in recognition of outstanding promise for a research career, will support up to two years of training with any of more than two dozen internationally recognized staff investigators working in modern facilities with state-of-the-art instrumentation. A partial list of research opportunities include the cell biology of cytoskeletal proteins, molecular biology or schizophrenia, receptors for steroid hormones and other endocrine hormones, cellular peptide growth factors, plasma membrane calcium pumps, computer modeling of protein structure and dynamics, optical NMR spectroscopic studies of biological molecular, macromolecules, biochemistry of Vitamin D, molecular genetics of clotting disorders and coagulation factors, regulation of protooncogenes and mechanisms involved in specific gene transcription. Salary and benefits will be determined by the successful candidate's experience but will be competitive with the salaries offered by the best fellowships available nationally at the Postdoctoral/Research Associate level. There is also an attractive package of benefits and an institutional allowance to support travel to scientific meetings.

Applicants should hold a doctoral degree in one of the life sciences or medicine with some research experience either in a formal graduate program or as a postdoctoral trainee.

Applications for this program should be submitted to Dr. Steve Brimijoin, Chair, Personnel Subcommittee of the Research Committee, Mayo Foundation, Rochester, MN 55905, by June 1, 1991.

Mayo Foundation is an affirmative action and equal opportunity educator and employer. (NW6697)E

UNIVERSITY OF NEWCASTLE UPON TYNE
DIVISION OF PATHOLOGY
MRC STUDENTSHIP

Applications are invited for a PhD studentship to work in one of the following areas: cancer research, human genetics, molecular biology, pathology, virology.

Applicants should possess or expect to obtain at least an upper second class Honours degree in biochemistry, genetics, immunology, microbiology, virology or related subjects.

Applications together with the names of two referees or enquiries for further particulars should be addressed to: Professor C.H.W. Horne, Division of Pathology, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP (Tel: 091 222 7144).

Closing date for applications: 17th May, 1991. (6193)F

FELLOWSHIPS continued

RESEARCH FELLOWSHIP

MOLECULAR APPROACHES TO ACTINOMYCETE ECOLOGY

A research fellowship, at postdoctoral or equivalent level, is available for a project on the biodiversity of actinomycete communities in natural environments using newly developed molecular strategies. The study will involve the use of PCR and rapid sequencing methods to study nucleic acids isolated from environmental samples, and the use of nucleic acid probes for the detection of target organisms.

The position is funded by Glaxo Group Research and will be based at the Natural History Museum, London, under the supervision of Dr. Martin Embley. There will be regular contact with scientists at Glaxo and Prof. Stan Williams at Liverpool University.

Candidates should have relevant practical experience in molecular biology, and one or two years' postdoctoral experience would be an advantage. The appointment will be for two years initially, extendable to three, and the starting salary is negotiable and dependent on age and experience.

For written details and an application form please contact:

Mr. Graham Aiston, Personnel, The Natural History Museum, Cromwell Road, London SW7 5BD. Tel: 071 938 9441.

Further enquiries about the position can be made to Dr. Embley at the Museum (071 938 8760) or to Dr. Divers or Dr. Vickers at Glaxo (081 422 3434 ext 2648 or ext 2330). The closing date for applications is 7th June 1991.

The Natural History Museum is an equal opportunities employer. (6210)E

THE NATURAL HISTORY MUSEUM

Glaxo Group Research

continued on pages 17 and 18

AWARDS

THE ORAL AND DENTAL RESEARCH TRUST

PROCTER & GAMBLE RESEARCH AWARDS

The aim of these awards will be to provide funds to facilitate research in an established unit in the UK.

Applications are invited from graduates already working in oral and dental research, particularly those interested in research related to plaque, gingivitis, caries, calculus and tooth hypersensitivity.

Applicants need not be graduates in dentistry and there is no limit of age, time since graduation or nationality. Consideration will be given to applications from research workers from overseas participating in joint work in the United Kingdom. The sum available for distribution in 1991-1992 is £15,000.

The closing date is 1st June 1991. There is no application form and applicants should set out the research programme in which they are engaged and the purpose for which funds are required with detail costing, together with a supporting letter from their head of department or supervisor. Four copies of each should be submitted. Applicants called for interview during June 1991 may apply for reimbursement of reasonable travelling expenses.

It is anticipated that the awards will take effect from the start of the 1991/92 academic year.

Applications to: The Director, The Oral and Dental Research Trust, Keats' House, 26 St Thomas Street, London SE1 9RN.

Procter & Gamble are the makers of Crest. (6082)N

PRIZES

PETER DEBIJE-PRIZE 1992 ON AGING OF THE BRAIN

The University of Limburg at Maastricht, The Netherlands, has been given the opportunity of awarding the Peter Debije-prize. This prize in the sum of 20,000 guilders is an expression of appreciation. The funds for the Peter Debije-prize are provided by the Edmond Hustinx Foundation in Maastricht. The prize is named after the physicist Peter J. W. Debije (1884-1966), a native of Maastricht, who was awarded the Nobel Prize for chemistry in 1936.

The prize will be awarded for the seventh time on January 10th, 1992 to a person or group of persons (three as a maximum, but preferably less than three) who are considered to have made a fundamental contribution to research in the field of Aging of the brain, particularly with respect to the (patho)physiology, epidemiology and clinical aspects.

The jury would like to draw the attention to persons or groups of persons from any country, who might be considered for this award on the basis of their scientific work in the field indicated.

Nominations (in English) should enclose a curriculum vitae, a survey of the achievements of the candidate(s) (not exceeding 4 pages) and a list of publications. Nominations, as well as questions about the award, should be addressed to the:

University of Limburg, att. dr. E.H.S. Drenthe, Secretary of the jury of the Peter Debije-prize 1992, Office of the Rector, P.O.0 Box 616, 6200 MD Maastricht, The Netherlands. Deadline for receipt of nominations is September 15th, 1991.

(W8457)U

Prix International de l'Association Française des Hémophiles Prix "Henri Chaigneau"

The International Prize of the French Association for Haemophiliacs whose aim is to encourage medical research into the disease will be awarded for the ninth time in 1992.

The previous winners have been:

★ 1979 A. L. BLOOM and I. R. PEAKE (Cardiff, UK)

★ 1981 P. M. MANNUCCI (Milan, Italie)

★ 1983 F. W. DAVIE and K. KURACHI (Seattle, Washington, USA)

and two medals awarded to L. W. HOYER (Connecticut) and E. G. D. TUDDENHAM (London)

★ 1985 J. M. NILSSON, M. BLOMBACK, A. AHLBERG, J. SOREFF and H. PETTERSON (Sweden).

★ 1988 H. R. ROBERTS (Chapel Hill, N. C., USA)

and two medals awarded to D. N. FASS (Mayo Clinic, Rochester, Minn., USA) and G. C. BROWNLEE (Oxford, UK)

★ 1990 C.A. FULCHER (La Jolla, California, USA)

and one medal awarded to K. NAFA (Alger, Algeria)

The regulations in French and English will be forwarded on request by the Secretariat of the selection committee (Association Française des Hémophiles - CNTS - 6, Rue Alexandre Cavanel - 75739 PARIS CEDEX 15). The work submitted for the Prize must reach the Secretariat of the selection committee by March 1st 1992 at the latest. (W8455)U

GLAXO INSTITUTE FOR MOLECULAR BIOLOGY

The Geneva Molecular Biology Research Institute of one of the world's most successful healthcare groups is offering a

POSTDOCTORAL FELLOWSHIP

The successful candidate will join a team working on the molecular basis of cell death in the vertebrate nervous system. Preference will be given to candidates with experience in primary cell cultures and recombinant DNA technology but interdisciplinary research is encouraged.

The Glaxo Institute for Molecular Biology carries out long term research to support the company-wide effort to discover effective new medicines. It provides a dynamic research environment and encourages the publication of scientific results as well as collaborative projects with academic groups.

We offer an excellent salary and benefits package and opportunities for career development in one of the most attractive parts of Europe.

If you are interested in this position, please send your curriculum vitae with a list of publications and the names of three referees to : Rita Gloor, Personnel Manager, GLAXO IMB S.A., 14, chemin des Aulx, Case Postale 674, 1228 Plan-les-Ouates, Geneva, Switzerland. (W8458)E

COURSES

INSTITUTE OF PSYCHIATRY
Department of Neuroscience
University of London

PHD NEUROSCIENCE

RESEARCH TOPICS INCLUDE

- Abnormal phosphorylation in Alzheimer's disease of the microtubule associated protein tau.
- Dopamine uptake and drug addiction studied using mammalian expression vectors.
- Effects of psychoactive drugs on gene expression in the brain.
- Effects of forebrain cholinergic lesions and of cholinergic rich transplants on sensitivity of rat cortical neurones to iontophoretic application of agonists.
- Environmental neurotoxins; their effects on neurons in culture.
- Glutamate receptors in schizophrenia studied by autoradiography in situ hybridisation.
- Localisation in human brain of genes expressed in schizophrenia.
- Long Term potentiation; the effects of drugs on its induction.
- Molecular genetics of the monoamine oxidases in psychiatric disorder.
- Neurotransmitter specific cell-surface molecules; structure and function.
- Optimisation of the cell lines for cognitively effective brain transplants.
- Platelet Ca^2 homeostasis and its abnormalities in Alzheimer's disease.
- Second messenger enzymes in schizophrenia studied by autoradiography and in situ hybridization.
- Role of phospholipase C in drug stimulated diacylglycerol formation.
- Sex steroids: their effects on dopaminergic neurons in culture.
- Signal transduction and the neuronal cytoskeleton.
- The effects of antidepressants on 5-HT receptors and receptor function.
- Prion proteins; biological function of normal and mutant human forms.

For further details, financial assistance, fees etc, please contact The Secretary, Department of Neuroscience, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF. Tel: 071 703 5411, ext. 3259. (6197)D

THE MARINE ENVIRONMENT AND BIOTECHNOLOGY
Ischia, 29 - 31 May 1991
Course Director: Gaetano Salvatore

Objectives:	To provide an overview of the aspects of molecular biology and biotechnology and of their application in the various areas of the marine environment.
Addressed to:	Young graduates or last-year undergraduates who work in the field of marine biology.
Program:	⁎ *Biotechnologies Today* ⁎ *Regulation of Gene Expression* ⁎ *Genetics in Acquaculture* Transgenic Animals and Transgenic Fish ⁎ *Molecular approach to the study of marine ecosystems*
Official language:	English
Location:	Hotel Regina Isabella - Lacco Ameno, Ischia (ITALY)
Deadline:	20th May
Registration Fee:	L. 900,000 (includes accomodation)
Organized by:	Stefania Ledda

For further information please contact:
Luisa Baggiani - Scuola Superiore di Oncologia e Scienze Biomediche - Via Passaggi 2 - 16131 GENOVA ITALY. Tel. 39-10-3774854/ 3774857; Fax 39-10-367618. (W8460)D

SCUOLA SUPERIORE DI ONCOLOGIA E SCIENZE BIOMEDICHE

Stazione Zoologica 'Anton Dohrn'

SCUOLA INTERNAZIONALE DI ONCOLOGIA BIOMEDICINA E SCIENZE AMBIENTALI

SENIOR RESEARCH FELLOW

MINERAL RESOURCES PROGRAMME – METALLOGENIC PROCESSES

A Research Fellowship is available in the Department of Mineralogy of The Natural History Museum, London, in the field of Metallogenic Processes.

The appointee will investigate the processes of element uptake, transport and deposition involved in the formation of metalliferous mineral deposits. This will include the application of chemical and fluid dynamic parameters to the study of ore mineral stability relationships in nature. The work will involve working as part of teams and may be interdisciplinary.

The Natural History Museum is internationally renowned for its collections and the research associated with them. The Department of Mineralogy is one of five scientific departments which are supported by the comprehensive library and full technical services. This Department holds the national collection of minerals, ores, meteorites and rocks and is well equipped for the study of these using modern spectroscopic, structural and analytical methods.

Applicants should have a good Honours Degree and a PhD in a relevant subject. The appointment will initially be for a fixed period of three years.

Starting salary, depending on qualifications and experience will be in the range £16,101 – £17,888

For further details and an application form, please contact: Miss Clare Beddall, Personnel Section, The Natural History Museum, Cromwell Road, London SW7 5BD Telephone: 071-938 8932 (an answer-machine will take your call outside normal office hours).

THE NATURAL HISTORY MUSEUM

The Natural History Museum is an equal opportunity employer.

(6180)E

GUNNAR NILSSON CANCER RESEARCH TRUST FUND

Grant applications up to 30,000 sterling will be considered to assist cancer research projects where money is not available from normal grant-giving bodies. There are no guidelines, any form of Cancer Research is eligible. Application forms are available from Dr Norman Howard, Chairman, Gunnar Nilsson Cancer Research Trust Fund, Department of Radiotherapy and Oncology, Charing Cross Hospital, Fulham Palace Road, London W6 8RF. 5 copies of completed applications which should be brief but adequate, are required by 27 July 1991.

The Trustees decisions will be made known in October 1991.

(6199)H

UNIDO

International Centre of Genetic Engineering and Biotechnology

ICGEB

GENETICALLY MODIFIED ORGANISMS: SAFETY IN THE LABORATORY AND THE ENVIRONMENT

Theoretical Course, 1-3 July, ICGEB Trieste, Italy

Topics:
Biological risk assessment;
Containment of GMOs in the field and the laboratory;
Monitoring methodologies for GMO field releases;
Comparative analysis of existing biosafety legislation;
Recommended procedures for safe practice;
Preferred host/vector systems;
Recombinants containing potentially oncogenic nucleic acid sequences;
Transgenic animals;
Databases and artificial intelligence in biosafety management.

Organiser: Gilbert Howe, Bristol, UK.

Lecturers:
Willy De Greef, Gent, Belgium
Peter Kearns, OECD, France
Terry Medley, USDA, Maryland, USA
Soren Molin, Lyngby, Denmark
Don Powell, Cambridge, UK
George Tzotzos, ICGEB.

The course is limited to 30 students.

The course is sponsored by the United Nations Environment Programme (UNEP)

GENETICALLY MODIFIED ORGANISMS FOR THE 1990's

Conference, 3-5 July, ICGEB Trieste, Italy

The Conference aims at being a forum for communication of the latest information on genetic engineering research, especially where this may soon lead to the production of organisms that could be released to the environment.

Topics:
Gene transfer and survival of microorganisms in the environment;
Environmental effects on the expression of cloned genes in plants;
Antisense RNA and fruit ripening;
Viral and bacterial vectors for vaccine development;
Baculoviruses as expression vectors;
Antisense RNA in viralcontrol;
Transformation of plants, fish and animals;

Organisers:
John Beringer, Bristol, UK George Tzotzos, ICGEB

Invited Speakers:
Raoul Barletta, Nebraska, USA: Chen Zhengliang, Beijing, China; Willy De Greef, Gent, Belgium; Don Grierson, Nottingham, UK; Peter Meyer, Cologne, FRG; Soren Molin, Lyngby, Denmark; Giovanni Paolella, Heidelberg, FRG; Marco Nuti, Padova, Italy; Nick Panopoulos, Berkeley, USA; Robert Possee, Oxford, UK; Don Powell, Cambridge, UK; Lucien Turay, London, UK; Luis Herrera-Estrella, Irapuato, Mexico; Jose La Torre, Buenos Aires, Argentina.

Closing date for applications to both the course and conference: 31 May 1991.

For further information about either please contact Ms Diana Viti, ICGEB, Padriciano 99, I-34012, Trieste, Italy. Tel: (39-40) 3757333 Fax: (39-40) 226555.

(W8454)D

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MEETINGS

EUROPEAN SYNCHROTRON RADIATION FACILITY

ESRF USERS' INFORMATION MEETING

Alpes Congrès Centre, Grenoble, France
Monday 8 July and Tuesday 9 July, 1991

ESRF is organizing a meeting to inform the synchrotron radiation user community about the state of preparations for experiments planned to start in September 1994.

The programme will consist of a series of talks mainly by ESRf staff followed by open discussion on topics such as

- parameters of machine, bending magnetis and insertion devices
- beamlines – planned parameters of optics, detectors & specimen containment
- computing at ESRF
- possible mechanism for beam-time allocation
- establishment of an EESRF Users' Organization.

A fee of 250 FF will be charged to cover the Abstracts, refreshments, and lunch on Monday 8 July. Attendees will be responsible for their own expenses.

Organizer: Professor A Miller

Applications to: Mrs Roselyn Mason
ESRF 02, BP 220, 38043 GRENOBLE Cedex, France
Tel: (33) 76 88 20 14; Fax: (33) 76 88 21 60

(W8461)S

MEDIA-SYSTEM

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9th - 12th October 1991

NEW STRATEGIES FOR IMMUNO-, TUMOUR- AND NEURO-THERAPY

An International Conference

Schloss Elmau
Garmisch-Partenkirchen, Germany

Our understanding of the molecular basis of physiological functions has accelerated dramatically through technical advances in molecular biology, and has led to new insights into the disorders underlying many diseases.

This is having a great impact on the development of a new generation of drugs and paves the way for new therapeutic regimens, based on our better knowledge of the mechanisms of drug action rather than on more or less serendipitous findings in 'classical' drug screening models. This conference will focus on progress being made in medicine through this approach.

Speakers will include:

● S Aaronson (Bethesda) ● M Allegretta (Stanford) ● K Beyreuther (Heidelberg) ● F Blasi (Copenhagen) ● C Boitard (Paris) ● D Capon (Cell Genesys, USA) ● J Collins (Cork) ● D Felten (Rochester) ● H Graeff (Munich) ● P Hellewell (British Bio-technology, Oxford) ● S Kaufmann (Ulm) ● R Morgan (Bethesda) ● M Pierotti (Milan) ● K Resch (Hanover) ● V Schirrmacher (Heidelberg) ● M Schreier (Sandoz, Basel) ● H Waldmann (Cambridge)

Attendance will be limited to 400.

Further details from:
Georgina Mason, IBC Technical Services Ltd.
Bath House, 56 Holborn Viaduct, London EC1A 2EX, UK.
Tel. 071-236 4080 Fax. 071-489 0849 (6225)C

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Announcing An International IBC Conference

ALZHEIMER'S DISEASE
Potential Therapeutic Strategies
24th/25th June 1991, London UK

By the year 2000, senile dementia will pose one of the greatest socio-medical problems yet faced by the Western world. As the proportion of elderly individuals within our population increases, so too will the incidence of a disease for which we have, at present, no effective treatment.

This international conference outlines the scope of the problem, and will be of interest to all working in the field of dementia, particularly on Alzheimer's disease.

To be Chaired by:
Dr Elkan Gamzu
Cambridge NeuroScience Research Inc, Massachusetts, USA
and Professor Raymond Levy
Institute of Psychiatry, London, UK

Speakers:

Dr M Rossor, St Mary's Hospital, London UK
Dr R. Dolan, The National Hospital of Neurology, London UK
Dr P. Luthert, Institute of Psychiatry, London UK
Dr E Gamzu, Cambridge NeuroScience Research Inc, USA
Dr G Konig, University of Heidelberg, Germany
Dr I Lieberburg, Athena Neurosciences, California USA
Professor R Mayer, Queen's Medical Centre, Nottingham UK
Dr A Kennedy, Hammersmith & St Mary's Hospital, London UK
Dr J D Wallace, Warner-Lambert Company, Michigan, USA
Professor B Costall, University of Bradford, UK
Dr U Schindler, Cassella AG, Frankfurt, Germany
Dr D Dawburn, Bristol Royal Infirmary, UK
Dr M Rogers, University of California, San Francisco, USA
Dr E Hall, The Upjohn Co, Michigan, USA
Professor J R M Copeland, The Institute of Human Ageing, UK.

For a full conference programme and registration details of the above event, please contact:

Renata Duke
IBC Technical Services Ltd
Bath House, 56 Holborn Viaduct
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(6185)C

MSc in MEDICAL GENETICS UNIVERSITY OF NEWCASTLE UPON TYNE

Applications are invited for one year full time MSc course in medical genetics being offered every year from October by Division of Human Genetics, School of Pathological Sciences in collaboration with the MRC Human Genetics Unit, Edinburgh.

This course includes extensive training in recent advances in clinical genetics and application of modern technology in medical and science research. The course is highly suitable for candidates qualified in medicine or dentistry or biological sciences.

This course is approved by the MRC and would fulfill the necessary genetic training requirement for a Clinical Geneticist, and provide academic/industrial career for scientists. One MRC Studentship is available.

Further details of the course may be obtained from Dr S.S. Papiha, Course Co-ordinator. Tel: (091 2226000, ext. 7461).

Application forms may be obtained from the Administrative Assistant, Medical School, University of Newcastle upon Tyne, UK. (6192)D

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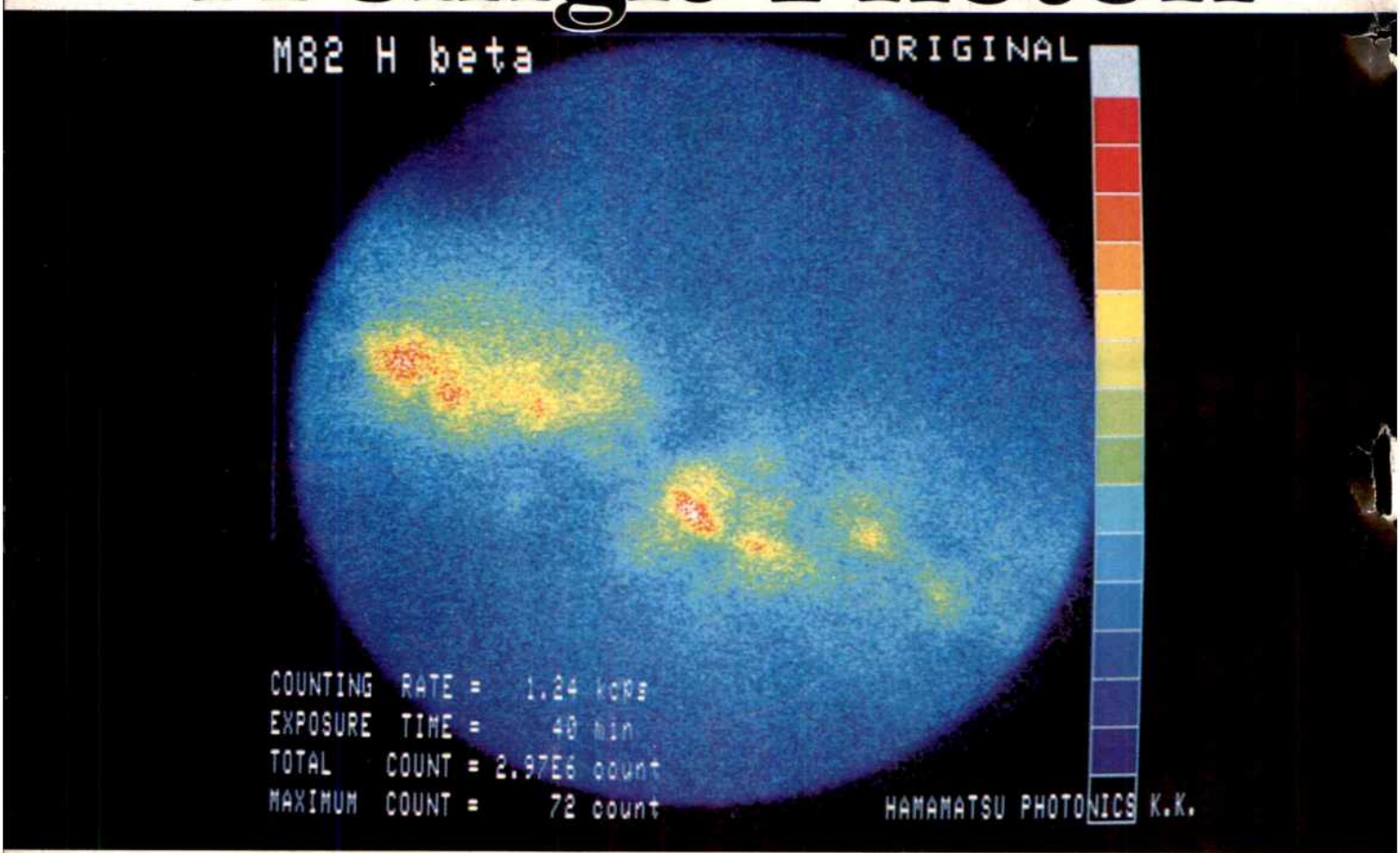
A Single Photon



This image of the center of the singular galaxy M82 was taken with the Hamamatsu Photon-counting Image Acquisition System, PIAS.
(Photo courtesy of the National Astronomical Laboratory; Kiso Laboratory of the University of Tokyo)

In the study of the behavior of photons we seek to comprehend the true nature of light. Photons behave like waves, but also like particles. Is it possible to define the nature of a single photon? What could such an achievement mean for science and humanity?

Research in photonics has yielded more than just paragraphs for textbooks. Our company's attempts to define the photon have brought unprecedented sensitivity to Hamamatsu photodetectors, which now have a wide range of practical applications. But this is only one example. Photonics offers a different viewpoint for unsolved problems in other fields of science as well.

At our Central Research Laboratory in Japan, research teams are studying how the findings from our investigations in photonics apply to molecular science, medical research communication, and many other fields.

As a world leader in light research, Hamamatsu Photonics not only strives to produce the most precise devices for light related research, but also to proceed on its own with basic research into the contradictions of the current understanding regarding the nature of light. We believe that working on the unsolved problems of photonics may lead to practical tools for a better life for all humanity and the achievement of world peace.

Teruo Hiruma

Teruo Hiruma
President, Hamamatsu Photonics K.K.

The photon is our business.

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